FLOWER BUD ABSCISSION AND ABSCISSION ZONES OF POTTED *Hibiscus rosa-sinensis*

By

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This dissertation is dedicated to my beloved parents, Abdullah and Maryam,
my husband Mohamed and my two daughters Sara and Laila

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FLOWER BUD ABSCISSION AND ABSCISSION ZONES ON POTTED *Hibiscus rosa-sinensis*

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Shipping potted flowering *Hibiscus rosa-sinensis* is a commercial problem due to abscission of flowers and flower buds. Several experiments were conducted to evaluate factors affecting bud abscission during shipping and the role of ethylene in this process. Plants were established in a greenhouse maintained between 26-30° C day and 21-24° C night under prevailing natural days. Flower buds were divided into six developmental stages: Stage 1 being the smallest (< 1 cm) and Stage 6 an open flower. At the beginning of each experiment, two flower buds of each stage were randomly chosen and tagged per plant before applying treatments. This was considered to be day 0 of the experiment.

To determine the effect of ethylene on flower bud abscission, two experiments were conducted. *H. rosa-sinensis* was very sensitive to exogenous ethylene, as flower bud abscission occurred when plants were exposed to 0.2-0.3 $\mu L.l^{-1}$. Larger buds were more sensitive to ethylene than the smaller ones. At low concentrations only the smaller buds (Stage 1 and 2) abscised prematurely while the remaining buds developed and senesced normally. Shipping temperatures and durations were also studied. High temperatures (25ºC) and longer shipping durations (4 and 6 days) caused greater bud abscission compared to lower temperatures (13 and 18º C) and shorter durations (2 days). During shipping, the smaller buds (Stage 1 and 2) abscised, while the larger buds developed and senesced normally.

A series of experiments were conducted using three ethylene inhibitors, Silver thiosulphate (STS), Aminoethoxyvinyl glycine (AVG) and 1-Methylcyclopropene (1-MCP), to determine the role of ethylene in bud abscission. Neither STS nor 1-MCP protected the plants from shipping damage, while AVG reduced bud abscission but did not prevent it. When shipping at lower temperature (20º C), regardless of duration, both STS and AVG prevented bud abscission. 1-Methylcyclopropene showed no transient effect on the plant; therefore once new receptor sites developed they were not protected from ethylene.

Experiments were conducted to study the anatomical mechanism of calyx and peduncle abscission zones. Observations showed the calyx abscission zone to be preformed, while the peduncle abscission zone only formed following induction. Removing the bud below the calyx abscission zone induced peduncle abscission zone formation, while removing the bud above the calyx abscission zone showed no effect. The peduncle abscission zone was not induced by ethylene. Results showed a dependent relationship between the calyx and the peduncle abscission zones.

CHAPTER 1
INTRODUCTION

The shipping of potted *Hibiscus rosa-sinensis* to retail markets without loss of quality poses a great challenge to both producers and researchers. During shipping, which may last for several days, plants may be exposed to temperature extremes, darkness and water stress. These adverse conditions, along with the duration of storage, may affect plant quality and reduce marketability. The most common consequences of shipping on *H. rosa-sinensis* are flower bud abscission and leaf yellowing. A limited supply of photosynthates causes premature flower abscission in most crops (Force et al., 1988). Low irradiance and darkness during the shipping may also induce abscission. Abscission under these conditions is thought to result from a low supply of photosynthates to the developing buds, and also a decrease in the ability of the flower bud to compete for the available assimilates (Brun and Brett, 1984; Heindle and Brun, 1983). Abscission of flower buds may also be induced by endogenous ethylene, produced due to stress during shipping or exogenous ethylene (Høyer, 1996), from smoke and pollutants.

Abscission is a physiological process whereby plants shed organs, or organ parts (Taylor and Whitelaw, 2001). Organs that abscise include leaves, fruits, flowers and flower parts such as buds, bud scales and others (Sexton et al., 1985). Abscission occurs as a normal feature of plant development with plant parts shedding in response to developmental cues, such as senescence (Sexton, 1995; Brown, 1997; Patterson, 2001). Plants usually shed damaged or infected organs as a defense mechanism to prevent the spread of infection (Patterson, 2001). Abscission may occur as a response to unfavorable environmental conditions such as temperature extremes, water stress and low light. Abscission may also be accelerated by ethylene (Sexton, 1995; Brown, 1997).

Abscission occurs at a genetically pre-determined abscission zone (Zanchin et al., 1995; Sexton, 1995), where the actual separation only occurs in a few cell layers, called the 'separation layer' (Brown, 1997). At the induction of abscission, cell wall-degrading enzymes such as cellulase and polygalacturonase are secreted, weakening the cell walls of the cells in the abscission zone (Sexton, 1995; Brown, 1997). The degradation of the cell wall is usually followed by mechanical force to facilitate the separation of the cells and the xylem (Sexton, 1997). The loss of structural integrity of the abscission zone can vary among the various organs of the same plant, as well as between similar organs in different species. Leaf and fruit abscission usually takes up to three days to complete whereas flowers and flower buds may abscise 1 to 4 hours after the induction of abscission (Sexton, 1995). The abscission zone can be created very early in the development of the organ, e.g., *H. rosa-sinensis* flower buds (Gilliland et al., 1976), or not until the induction of abscission, e.g., cotton leaves (Dutt, 1928). The cells of the abscission zone are created by continuous cell division in the region (Halliday and Wangermann, 1972; Gilliland, 1976). However, abscission in some species is not preceded by cell division, e.g., *Impatiens* (Sexton, 1976) and cotton (Dutt, 1928). Ethylene was found to accelerate abscission by inhibiting cell division in the abscission zone (Gawadi and Avery, 1950).

In many studies ethylene has been implicated in acceleration of abscission, however, it may not be the only regulator of abscission (Brown, 1997). Abscisic acid has also been implicated in abscission (Panavas et al., 1998). Ethylene has been shown to induce abscission of a wide variety of organs and in many species (Sexton et al., 1985). Both ethylene concentration and exposure duration affect abscission (Taiz and Zieger, 1998; Sexton, 1997). Typical exogenous ethylene concentrations commonly range between 0.1 and 5 μL/L to exhibit an abscission response (Sexton et al., 1985). The sensitivity of a plant to ethylene varies depending on the levels of receptors in the organ (Aloni et al., 1994; Høyer, 1996; Sexton, 1997). Some other factors that increase sensitivity to ethylene are water stress, aging and ethylene autocatalytic reaction (Sexton et al., 1985).

Compared to other potted flowering plants, *H. rosa-sinensis* is very sensitive to ethylene (Høyer, 1996). Høyer (1996) demonstrated that an ethylene exposure of concentrations as little as 0.05 μL.l for 24 hours caused flower bud abscission. Extensive flower bud abscission in *H. rosa-sinensis* can also be caused by darkness or low light conditions (Høyer, 1996; Force et al., 1988).

Treatment with an ethylene action inhibitors such as silver thiosulphate (STS) was effective in reducing ethylene-induced bud abscission as well as dark-induced abscission (Thaxton et al., 1988). Reid et al. (2002) reported that 1-aminocyclopropene (1-MCP) did not extend the flower life of *H. rosa-sinensis.* There are no reports that focused on the treatment of plants with 1-MCP prior to dark shipping. There have been no reports on the effect of aminoethoxyvinyl glycine (AVG), an ethylene biosynthesis inhibitor, on *H. rosa-sinensis.* However, it has been reported that AVG treatment delayed abscission of cocoa flowers (Aneja et al., 1999) and Christmas cactus (Serek and Reid, 1993).

The primary objective of this dissertation was to determine the best methods for shipping potted flowering *H. rosa-sinensis* plants by minimizing the damage caused by the shipping environment. More specifically, the effect of shipping on the pattern of bud abscission of the cultivar ‘Pink Versicolor’ was evaluated. Several ethylene inhibitors including AVG, 1-MCP and STS were used to help elucidate the role ethylene in floral bud abscission. Trials were conducted to evaluate the effectiveness of anti-ethylene inhibitors on different stages of bud and flower development. Anatomical changes of the calyx and peduncle abscission zones of *H. rosa-sinensis* during shipping and after ethylene exposure were also studied to gain insight into the mechanism of the abscission zones.

Premature floral abscission is a problem that affects many agricultural and horticultural crops. Therefore, understanding the responses of the plants to the effectors causing floral abscission will allow greater perspective on how to reduce the problem. The capacity to understand and prevent *H. rosa-sinensis* bud abscission will improve the postproduction of this crop. The results of our work will probably be of greatest interest and importance to the floriculture industry.

CHAPTER 2
LITERATURE REVIEW

Hibiscus rosa-sinensis

Hibiscus rosa-sinensis, a member of the Malvaceae family, is grown widely in the tropics and is believed to have originated in China, Vietnam and the East Indies, but its exact origin is not known. Cultivation of *H. rosa-sinensis* started from the dawn of history in China and now there are about 250 species distributed geographically but mostly in the tropics (Wilkins, 1998). In the early nineteenth century, more forms of *H. rosa-sinensis* were introduced to greenhouses in Europe. Most of the plants were from Asia and islands of the South Indian Ocean. Breeding of old *H. rosa-sinensis* with other native *Hibiscus* started as early as 1820 by Charles Telfair (Howie, 1980). However, the most significant interest in breeding *Hibiscus* species and hybrids began around 1900 in Hawaii, India, Fiji, Ceylon and Florida, where the primary interest is today. All forms of *H. rosa-sinensis* found today are believed to have evolved from inter-specific hybrids using species, of genetic compatibility such as *H. schizopetalus*, an African east coast species; *H. liliiflorus* from Mauritius and Rodriguey Island and many more (Howie, 1980).

There is a possibility that among the many varieties of *Hibiscus* currently considered under *H. rosa-sinensis* are some that are true species. The solitary flower of *H. rosa-sinensis* is a simplified inflorescence presumably reduced from a more primitive inflorescence, which contained several pediculate flowers on a single stalk (Gilliland et al., 1976). Some species of *H. rosa-sinensis* as well as other Malvaceae have compound inflorescence with each flower having an abscission zone at its base (Bates, 1965).

H. rosa-sinensis is a very popular perennial used both as an indoor plant in the northern cooler regions and as an outdoor shrub in semi-tropical and arid regions (Wilkins, 1998). There is great potential in the marketing of Hibiscus as a flowering potted plant. However, shipping potted Hibiscus is commercially difficult due to abscission of flowers and buds, as well as leaf yellowing either during (Rasbach, 1985) or after shipping (Gibbs et al., 1989).

Senescence and Abscission

Plants have a unique ability to abscise entire, functioning organs as a part of their life cycle (Panavas et al., 1998). The period preceding organ abscission, known as senescence or aging, involves active processes that are induced either by developmental stage, environmental factors, or both (Nooden and Guitamet, 1996). During senescence, specific genes are stimulated by endogenous signals, and these signals can indirectly initiate degradation of proteins, RNA, lipids and chlorophyll (Buchanan-Wollastan, 1997). Breakdown and relocation of essential cellular components are the first stages of the senescence process. This is followed by destruction of membranes and finally cell death in the senescing organ (Panavas et al., 1998). Typically senescence of plant organs is followed by abscission (Addicot, 1982). The latter process takes place in the leaf petiole or floral peduncle, in which specific cells differentiate to form an abscission zone. This allows the senescent organ to abscise from the plant.

Most studies on senescence and abscission have focused on leaves. There has been extensive research on physiological, biochemical and molecular aspects of leaf abscission. Leaf abscission is a normal feature of plant growth as the older leaves become shaded by the canopy and lose their capacity to provide enough carbon to the growing plant (Brown, 1997; Gan and Amasino, 1997). Abscission provides an advantageous means of shedding older leaves before their use of water and nutrients exceeds their carbon fixation (Brown, 1997). However, in agricultural crops, leaf abscission may limit the crops' yield, as well as contribute to postharvest losses in vegetables. Thus, this process is not necessarily desired (Gan and Amasino, 1997). The leaf abscission zone ages and becomes sensitive to ethylene, which is thought to promote abscission and accelerate the process. Other studies indicate that ethylene is not the only factor regulating leaf abscission (Abeles et al., 1992; Brown, 1997; Gan and Amasino, 1997).

Floral senescence and abscission have been extensively studied in many agricultural and horticultural crops to minimize the damage caused by untimely loss of buds, flowers or immature fruit. Evidence supports a major role for ethylene in the control of floral abscission, and it does appear to be the primary regulator of the abscission process in many plants (Goodspeed et al., 1918; Sampson, 1918; Arrillaga and Jones, 1944; Brown, 1997; Taiz and Zieger, 1998). In many species, the corolla wilts before the flower parts drop, and the progression of these events varies for different plant families (Woltering and Van Doorn, 1988). However, flowers that abscise rather than wilt tend to exhibit the greatest ethylene sensitivity (Brown, 1997). Flowers that are very sensitive to external ethylene during development or at pollination also appear to be very responsive to endogenously produced ethylene (Brown, 1997).

Other factors have also been reported to accelerate abscission of various plant organs (Gawadi and Avery, 1950) such as water deficit (Sampson, 1918; Haas, 1927), extreme temperatures (Lloyd, 1914), insufficient light (Dunlap, 1943), illuminating gas (Zimmerman et al., 1930), carbon dioxide, chloroform and nutrient deficiency (Mason, 1922), insect injury and fungal attack (Coit and Hodgson, 1919).

Abscission and the Abscission Zone

Abscission is a mechanism used by the plant to shed organs or part of organs (Zanchin et al., 1993; Zanchin et al., 1995; Sexton 1994). It is involved in the shedding of leaves, fruits, flowers and floral parts (Sexton, 1994). Abscission involves physiological activity in specialized cells within an abscission zone (Addicot, 1982). Abscission occurs precisely in a narrow section, within the abscission zone, that is a few cell layers thick and called the separation layer (Brown, 1997). The cells within the separation layer are alive and utilize the energy of metabolism for synthesis and secretion of hydrolytic enzymes (Addicot, 1982). This active metabolic process results in a weakening of 1 to 20 rows of cells (Sexton, 1994).

The physiological process of abscission and rate of abscission vary among organs and species (Addicot, 1982). Effective secretion of hydrolytic enzymes will result in a rapid and clean separation. Nevertheless, some species show little indication of hydrolytic activity, but the tissue at the abscission zone is weak and less lignified. Therefore, these abscission zones are more susceptible to attack by microorganisms and to mechanical separation (Addicot, 1982). In the abscission zone, the hormonal influences of the organ interact with influences from the rest of the plant, such as nutrient levels. The balance of hormonal influences at the abscission zone provides the information that tells the cells whether they should become active and abscise the organ (Addicot, 1982).

The position of the abscission zone is a genetic characteristic of the species (Sexton, 1995). In most cases, the abscission zone is not inherently weak and a process leading to weakening of the abscission zone causes the shedding of the organ. In beans, 0.5 kg can be hung on the leaf abscission zone without causing a split of the zone; however, after abscission is induced, the same abscission zone will weaken and can break at the slightest touch (Sexton, 1995). In the weakening process, cell wall breakdown is the main factor causing the separation of the intact cells (Sexton and Roberts, 1982). The cell walls of the abscission zone are believed to be enzymatically degraded prior to abscission. However mechanical forces are required to facilitate separation of the xylem (Sexton and Roberts, 1982). The speed of abscission also varies. Abscission of leaves and fruit usually takes 12-48 hours (Sexton et al., 1989; Brown, 1997) whereas petals (Evensen et al., 1993) and flower buds (Sexton and Roberts, 1982; Roberts et al., 1984) may take only a few hours.

Abscission zone anatomy contains subtle characteristics that distinguish the region from adjacent cell layers (Sexton and Roberts, 1982). Cells of the abscission zone are smaller in size (Zanchin et al., 1995; Huberman et al., 1983; Oberholster et al., 1991) and densely packed, which makes them darkly pigmented when viewed externally (Sexton, 1995). Flower abscission zones are made up of small, cytoplasmic cells, which show longitudinal divisions (Evensen et al., 1993; Mckenzie and Lovell, 1992). The abscission zone is anatomically distinct from other cells in the amount of cytoplasm, starch content and cell lengths (Gilliland et al., 1976; Oberholster et al., 1991). The small cells of the abscission zone are created by constant cell division in the region (Halliday and

Wangermann, 1972). On the other hand, there is no evidence of cell division in the abscission zone of cotton flower (Dutt, 1928).

Physiologically, abscission zone cells are the only ones capable of responding to active stimuli that lead to the loss of adhesion between cells and then the shedding of the organ (Zanchin et al., 1995; Brown, 1997). In the abscission zone, sclerenchyma fibers are often replaced by collenchyma cells that lack lignification. The lack of lignification facilitates separation (Sexton, 1995). The close-packed cells of the abscission zone are angular, but expand and become round in shape as the cell walls degrade (Sexton and Redshaw, 1981). The increase in size and rounding is assumed to cause the force that ruptures the xylem (Sexton and Redshaw, 1981).

According to Huberman et al. (1983) the pattern of abscission may occur in several ways: it may occur from the surface groove around the abscission zone, through the cortex and the vascular cylinder and into the pith as in the abscission zone of citrus (Jaffe and Goren, 1979); in the bud-peduncle of *Begonia* (Hanishten Cate et al., 1975); in the peduncle of *H. rosa-sinensis* (Gilliland et al., 1976), or in the pedicel of soybean (Oberholster et al., 1991). Abscission may also start from the inner cortex and progress outward to the epidermis as in olive fruits and leaves (Reed and Hartman, 1976). In emasculated flowers of tomato, the abscission zone consisted of 6-8 layers of disk-shaped cells (Tabuci et al., 2001). As a result of cell enlargement in the epidermis, cortex, inner vascular bundle and central parenchymatous region of the pedicel, large intercellular cavities formed causing the flower to abscise 14 days after emasculation (Tabuci et al., 2001).

As previously addressed, the separation process involves a narrow layer of cells across the abscission zone known as the separation layer (Sexton, 1995). The cells of the separation layer do not differ from the other cells of the abscission zone. However, the cell walls degrade in the separation layer during abscission, while identical cells on either side remain intact (Sexton, 1995). One hypothesis suggests that cells of the separation layer are biochemically distinct compared to the rest of the abscission zone cells (Osborne, 1991). Another hypothesis assumes all the cells have the potential to degrade the cell wall, but that the abscission signal triggers only a few rows (Sexton and Roberts, 1982). The separation layer is made up of living cells, which utilize the energy of metabolism for the synthesis and secretion of hydrolytic enzymes (Addicot, 1982).

Abscission zones can be formed very early in the development of the organ as in *H. rosa-sinensis* (Gilliland, 1976); however they are not necessarily active throughout the life of the organ (Sexton, 1995). The abscission zone can be activated in response to a specific trigger at one stage of development and the same zone can become inactive and not respond to an identical stimulus at a different stage (Greenberg et al., 1975). Peach flowers have three abscission zones (Rascio et al., 1985) that are each activated at different developmental stages of the flower. It is believed that the loss of abscission zone responsiveness is caused by a loss of some vital component such as hormone receptors (Sexton, 1995). Another explanation is that the abscission zone responds to the stimuli but the cells of the separation layer are unable to separate because they are modified by lignin or suberin, which make them resistant to hydrolase attack (Sexton, 1995).

Induction of abscission is normally a part of the senescence program following the yellowing of leaves and ripening of fruits. However, abscission can take place in immature organs in the absence of senescence (Sexton, 1995). Pollination can dramatically accelerate petal abscission (Halevy et al., 1984). Bud abscission is a serious problem in commercial crops. Enhanced bud abscission has been reported due to diseases, water stress, temperature extremes and mechanical shaking (Sexton, 1995).

The mechanism of abscission has been studied widely since the early 1800's due to its effect on commercial crop production. Early beliefs were that abscission is caused by the formation of a corky layer on the stem side of the abscission zone cutting the supply of nutrients and water in the separation layer cells leading to collapse of the cells. However, Inman (1848) suggested that the cells of the separation layer are living during abscission and Von Mohl (1860) found that abscission would take place without the formation of epidermis.

Two theories were proposed more recently to explain abscission. The turgor theory was that starch degradation causes an increase in the solute concentration in the separation zone. The increased turgor pressure causes the cells to become round and the wall to tear along the middle lamella (Sexton, 1995). The second theory was that not all cell separation begins at the corner of the cell and some separating cells do not change from angular shape to round (Kendall, 1918).

After the induction of abscission, several anatomical changes take place. In many species, one of these changes is an increase in cell division in the region of the separation layer (Sexton and Roberts, 1982). In *Impatiens* (Sexton, 1976) and cotton pedicels (Dutt, 1928), however, abscission is not preceded by cell division. It has also been shown that cell division is absent if ethylene is used to accelerate the abscission as reported in Poinsettia leaf abscission; however, cell division does occur during normal abscission (Gawadi and Avery, 1950). At present, there is a general belief that abscission involves the induction of wall-degrading enzymes, which weaken the cell walls of the abscission zone (Horton and Osborne, 1965; Sexton and Roberts, 1982; Brown, 1997). The most common enzymes associated with abscission include cellulase and pectinase (Sexton et al., 1985, Brown, 1997). During abscission, cell wall hydrolysis is visually evident, especially in the middle lamella (Evensen et al. 1993; Oberholster et al., 1991; Sexton et al., 1983). In the flower pedicel abscission zone of Begonia, soluble pectins accumulated in response to abscission (Hänisch Ten Cate et al., 1974).

The exact role of the various enzymes involved in abscission has not been established (Brown, 1997). The difficulty is mainly due to the separation layer which is only a few cell layers thick and makes biochemical studies difficult (Sexton et al., 1985). In addition, there are many different cellulases, pectinases and other cell wall degrading enzymes in the plant, but only certain isoforms of these enzymes are involved in abscission (Brown, 1997). The increase in certain isoforms of cellulase has been reported in some abscission systems including leaves (Sexton et al., 1980), flowers (Roberts et al., 1984) and fruits (Bonghi et al., 1992). Addicot (1982) suggested that cellulase activity is not involved in separation but rather the wound-healing response that follows abscission. According to Sexton (1976), cellulase is involved in generating the mechanical force required to break the abscission zone. Huberman et al. (1983) observed that abscission zone cells in citrus fruit follow a series of events that lead to cell separation, including swelling of cell walls, a gradual disappearance of cell wall polysaccharide component, cell wall dissolution and degradation, and finally free protoplasts in the starch sheath area where the specific group of cells were organized. They sometimes also observed cell expansion in the starch sheath region of the abscission zone in which starch grains had accumulated earlier. An increase in starch was also found in the chloroplasts of cells proximal to the separation layer of *H. rosa-sinensis* pedicle (Gilliland et al., 1976).

Hibiscus Abscission Zone

Gilliland et al. (1976) studied the ultrastructure of the pedicel (calyx) abscission zone in *H. rosa-sinensis* to develop a better understanding in the mechanism of abscission. Samples of pedicles were collected (1) early, 2.5 weeks before anthesis, (2) at anthesis and (3) late, 1 week after anthesis. They found that the pedicle abscission zone of *H. rosa-sinensis* was apparent at the early developmental stage. It was characterized by a narrow constriction and dark discoloration. The development of the separation layer was 3 or 4 cells distal to the abscission zone constriction and included the 2 or 3 most distal tiers of dividing cells of the abscission zone. Cell division continued up to the time of abscission. The protective layer formed immediately proximal to the separation layer. Cells on both sides of the separation layer became elongated and different in size compared to the remaining cells in the abscission zone.

Gilliland et al. (1976) also found that the vascular tissue at the pedicel abscission zone was less developed than in neighboring tissue. Mucilage canals were present in the cortex and pith proximal to the abscission zone; however, they did not occur across the abscission zone into the distal pedicel. Relatively little starch was found in the cells at the early sample time; however, by anthesis a considerable amount was deposited in the chloroplasts of the cells proximal to the separation layer and a smaller amount in the distal region. Numerous crystals believed to be calcium oxalate were observed in parenchyma cells of the abscission zone at all three sampling stages. As abscission approached the number of crystals increased in correlation with the degradation of pectic substances. The calcium released by the hydrolysis of cell wall pectins was thought to have contributed to increased deposition of calcium oxalate in druses.

Separation of the pedicle abscission zone started at the periphery of the separation layer and proceeded centripetally toward the pith. The hydrolytic activity during abscission, including the time of separation, appeared to involve only the middle lamellae and cell walls, whereas the protoplast remained intact.

Electron microscopy revealed that the organelles of the cells in the separation layer for the early sample stage showed few changes until anthesis and the approach of abscission. The changes in the number and nature of the cytoplasmic organelles were found to be relatively small as the abscission zone progressed. Results confirmed the observations from light microscopy: that the protoplasts of the cells of the separation layer remain intact and appear active until the separation is completed.

After separation, the remaining abscission zone cells became active and secreted special wall substances of the periderm protective layer. Major changes were mainly found in the middle lamellae and cell walls. Therefore, cells of the subtending separation layer did not show ultrastructural disintegration that occurred in senescing tissues. Pectinases and cellulases were produced in larger amounts as abscission approached probably causing the hydrolysis of the middle lamella. This study gives a clear insight to the process of abscission in calyx abscission zone of *H. rosa-sinensis*.

Hibiscus and Shipping

Bud abscission during shipping of *H. rosa-sinensis* is a serious commercial problem that limits its use. There have been a few reported studies to identify the causes of the problem. Nell and Barrett (1984) described the effect of shipping temperature and duration on pre-finished flowering *H. rosa-sinensis*. Using the cultivars 'Red American Beauty' and 'Seniorita', they placed the plants in paper sleeves and wax-lined boxes before putting them in environmentally controlled chambers. After treatment, the plants were removed from the boxes and sleeves and placed in a greenhouse. In one experiment, plants were kept for 2, 4 or 7 days at 5, 16 or 27° C and the number of buds present was determined after 5 days in the greenhouse. The two cultivars responded similarly to shipping temperatures and duration. A shipping duration of 7 days at the low and high temperature caused the most bud abscission. In a second trail, they evaluated the effect of bud size on susceptibility to drop during simulated shipping at 27° C for 2 and 4 days. Most buds abscised on plants held at 27° C regardless of the bud size at the time of treatment. Two to four times as many smaller buds (less than 1.27 cm in length) abscised, as did buds greater than 1.27 cm. They showed that although the 2 days shipping duration left the plants with more intact buds, abscission progressed rapidly following removal from the boxes. They concluded that shorter duration (less than 4 days) and optimum temperature (around 16° C) were conditions conducive to maintaining buds during shipping.

Thaxton et al. (1988) also studied the effect of shipping temperature on flower bud abscission of *H. rosa-sinensis*, cultivar 'Brilliant Red'. They divided the flower buds into four stages of maturity: Stage 1 (> 4 cm length with corolla extended beyond calyx), Stage 2 (3-4 cm with pigment corolla visible), Stage 3 (2-3 cm with no corolla visible) and Stage 4 (< 2 cm length with calyx not parted). Their first experiment was conducted to determine the effect of storage temperature on bud abscission. Plants in closed plastic bags with ventilation holes to allow gas exchange were placed in chambers at 10, 20 or 30° C for 4 days. At 30° C there was about 10-fold greater bud abscission than at 10° C, showing that high temperature increased bud abscission. There was no effect of shipping temperature on bud stages. In another experiment, plants were sprayed with 0 or 4 mM STS before being placed in paper sleeves and then stored in the dark for 4 days at 10, 20 or 30° C. After removal from storage, the plants were placed in a simulated office environment and bud abscission was monitored. STS at 4 mM was found to be effective in reducing dark-induced bud abscission at all temperatures compared to control plants. STS completely prevented bud abscission on plants shipped in dark at 20° C for 4 days then kept in the interior environment compared to plants shipped at 10 or 30° C where approximately 7 % of the buds abscised.

In a similar study, Force et al. (1988) characterized *H. rosa-sinensis* bud abscission induced by dark storage. They also used 'Brilliant Red" in their experiments where plants were placed in a controlled environment for 5 days under complete darkness at 20 ± 2° C and 50 to 60 % R.H. Following the dark treatment the plants were returned to the greenhouse for observation. They divided the buds into three different developmental stages: very young buds (< 10 mm), expanding buds (10 – 30 mm) and mature buds (> 30 mm). Results showed that dark storage for 5 days promoted flower bud abscission and that the percentage of bud abscission increased during the post storage observation period. They found that by day 10, 93 % of buds had abscised. They also found that the most mature buds were least likely to abscise during dark treatment and often opened during or after the dark storage. Less developed buds showed higher rates of abscission following the dark treatment. From these results they proposed that removal of older non-abscising buds would reduce the extent of bud abscission on younger buds. To test this hypothesis, flower buds larger than 30 mm were removed before the dark treatment. Removal of these buds resulted in a significant reduction in abscission of younger buds.

Van Meeteren and Van Gelder (1995) studied the involvement of ethylene in *H. rosa-sinensis* bud abscission and the role of flower buds in causing dark induced flower bud shedding due to pedicel abscission. In this study, they used the cultivar 'Nairobi' and in all the experiments plants had at least 3 flower buds between 5 and 10 mm with larger buds removed prior to treatment. Plants were placed in growth chambers at 20° C and either kept in complete darkness or light for 12 hrs/day. Plants kept in the dark for 3 days started to abscise their flower buds due to pedicle abscission, whereas plants kept under light had no abscission at all. They also found that flower buds from plants exposed to dark treatment showed an increase in ethylene evolution with exposure time. This increase in ethylene was absent in light-treated plants. They found that explants kept in the dark had 50% abscission after 4 days and no abscission in light-treated explants. Results for the ethylene part of the experiment were not reported.

Høyer (1986) studied the relationship between bud abscission in *H. rosa-sinensis* during simulated shipping and ethylene by using STS. Plants were sprayed with different concentrations of STS ranging between 0.05 and 4.05 mM. The plants were sprayed 1, 11 or 21 days prior to the shipping treatment. Plants were placed in the dark for 72 hours at 21° C (simulated shipping) and after the treatment the plants were placed in an interior environment for observation. Results showed that 72 hours of darkness caused a constant drop of buds during the observation period. Buds showing color were more sensitive to the dark treatment than less mature buds. Spraying plants with STS 1 and 11 days before the dark treatment reduced bud drop whereas spraying 21 days before was ineffective. Also, higher concentrations of STS provided better inhibition of abscission. Spraying plants 1 day before shipping resulted in fewer drops of younger buds (green) buds than when sprayed 11 days before shipping.

In 1996, Høyer reported the results of another study focusing on the effects of ethylene concentrations on *H. rosa-sinensis* held in the dark. Plants were exposed to 0.0, 0.05, 0.1, 1.0 or 5.0 $\mu l\ l^{-1}$ of ethylene during the last 6, 12, 24 or 72 hours of the dark period. Data recorded were the number of flowers, younger buds (green) and buds showing color and the abscission of buds and flowers. Høyer (1996) found that the dark treatment without ethylene increased bud abscission compared to plants held in the greenhouse. Interestingly, he reported that low ethylene levels delayed abscission and with increasing ethylene exposure the delay was reduced. The results showed that abscission of buds is induced by ethylene with mature buds (showing color) being more sensitive to ethylene and total abscission at lower ethylene exposure compared to less mature buds (green).

From the work that has been reported bud abscission during shipping of *H. rosa-sinensis* can be caused by low light conditions (Nell and Barrett, 1984; Force et al., 1988; Thaxton et al. 1988; Gibbs at al., 1989; Van Meeteren and Van Gelder, 1995; Høyer, 1986, 1996) and external ethylene (Høyer, 1996). Ethylene has been implicated in inducing bud abscission during dark treatment as reported by Høyer (1996). However, the role of ethylene during abscission is still not clear due to discrepancies found in the literature.

Auxin and Abscission

The role of auxin in abscission was documented at the beginning of the century when the removal of the leaf blade induced the weakening of the abscission zone in the leaf petiole of bean (Sexton, 1997). This led to the belief that the actively growing leaf produces an inhibitor, which moves down to the petiole and prevents the induction of the abscission zone (Addicot, 1970; Sexton, 1995). Application of auxin to the petiole was found to prevent abscission, which resulted in the proposal that IAA was a natural inhibitor of abscission (Sexton, 1997). The theory of inhibition of abscission by auxin was supported by the correlation between declining levels of auxin and the occurrence of natural abscission (Addicot, 1970; Sexton, 1995, 1997). The levels of the auxin were found to drop rapidly as leaves yellowed and abscission approached (Addicot, 1970). There is a general acceptance that auxin retards or prevents abscission if the auxin levels in the abscission zone remain above a critical level (Cracker and Abeles, 1969; Beyer and Morgan, 1971; Addicot, 1982; Sexton, 1995, 1997). When an IAA flux is maintained at the abscission zone, cell separation is inhibited and abscission is prevented (Addicot, 1982; Sexton et al., 1985). The amount of auxin reaching the abscission zone and the distribution of auxin on both the proximal and distal sides of the abscission zone appear to be an important factor in the preventive action of auxin (Beyer and Morgan, 1971). Rate of auxin transport to the abscission zone from the distal organ, biosynthesis and metabolism of auxin are important factors in determining the level of IAA in the abscission zone (Sexton, 1995; Taylor and Whitelaw, 2001). The direction of auxin gradient across the abscission zone is also important whereas the absolute concentration is not a determining factor on abscission (Addicot et al, 1955; Brown, 1997).

Cracker and Abeles (1969) showed that a decrease in break strength of bean explants was prevented when IAA was applied shortly after the excision. However, when IAA application was delayed, there was a significant reduction in its effectiveness. However, Rubinstein and Leopold (1963) found that adding auxin 12 h after deblading leaves accelerated abscission instead of preventing it. Based on their findings Rubinstien and Leopold (1963) brought forward the assumption that explants have two stages of responses to auxin. In stage 1, the addition of auxin would prevent abscission and prolong the stage. However if the level of auxin fell, stage 2 was entered and abscission was accelerated instead. The opposing effect of auxin in stage 2 was later explained by the relationship between ethylene and auxin (Sexton, 1995; Brown, 1997). The duration of stage 1 is dependent on the auxin level in the abscission zone tissue (Sexton and Roberts, 1982), and the level of auxin in the abscission zone controls the sensitivity to ethylene (Taylor and Whitelaw, 2001). Auxin prevents the abscission zone from becoming sensitive to ethylene (Addicot, 1982), and it can determine the time at which the abscission zone becomes sensitive to ethylene (Jackson and Osborne, 1972). The abscission zone becomes sensitive to ethylene as the auxin flux from the leaf declines with age (Brown, 1997). Ethylene is a potent inhibitor of auxin and it may participate in abscission through its effect on auxin transport and synthesis as well as enhancement of auxin destruction (Beyer and Morgan, 1971; Beyer, 1973; Sexton et al., 1984). Ethylene has been shown to have both indirect and direct effect on auxin moving from leaf blades and the base of the petiole abscission zone (Beyer, 1975). However the balance between auxin and ethylene appears to be of major importance for the initiation of abscission (Taylor and Whitelaw, 2001).

Abscisic Acid and Abscission

Abscisic acid (ABA) was found to play a role in abscission along with auxin and ethylene (Cracker and Abeles, 1969). However, the role ABA in regulating abscission was found to be minor (Patterson, 2001) or less certain compared to ethylene and auxin (Suttle and Hultstrand, 1993).

In a study of *H. rosa-sinensis* abscission zone, Swanson et al., (1975) found increased ABA levels at the time of actual separation of the calyx abscission zone. ABA levels increased in the young fruit at the time of abscission, and it was found to promote abscission when applied to the young cotton fruit (Addicot, 1982). ABA levels also increased significantly in flowers of cocoa prior to abscission (Aneja et al., 1999). Increasing amounts of ABA caused an increase in ethylene production, and a close correlation was found between the loss of break strength in bean and ethylene produced due to ABA (Cracker and Abeles, 1969). Muller at al. (1999) studied the involvement of ABA in shelf life of miniature-potted roses and found that exogenous application of ABA accelerated flower senescence. ABA treatment increased ethylene production in one of the cultivars, while no ethylene was measured in others studied. In this study, they concluded that the effect of ABA is at least partly mediated by ethylene since pre-treatment with 1-MCP delayed senescence. Panavas et al. (1998) found that flowers of daylily were insensitive to ethylene, whereas exogenous ABA promoted senescence.

While the exact role of ABA in abscission is not clear, there are two contrasting roles proposed (Suttle and Hultstrand, 1993). The first considers ABA as a major hormone in regulation of abscission with a yet unidentified role (Addicot, 1983), and the second proposed role is indirect and mediated by changes in ethylene (Sexton and Roberts, 1982; Morgan, 1984). ABA was found to promote abscission when applied during stage 2 of response to auxin, as it is believed to reduce IAA transport, however ABA does not shorten stage 1 of response to auxin (Sexton and Roberts, 1982).

ABA appears to potentially play an essential role in ethylene-induced abscission (Suttle and Hultstrand, 1993). Ethylene accelerated abscission in cocoa flowers but only in the presence of ABA (Aneja et al., 1999). Muller et al. (1999) suggested that ABA and ethylene interact during senescence and abscission with the effect of ABA being partly mediated by ethylene. The mechanism for ABA action may involve reducing auxin levels by increasing senescence rate of the leaf (Jackson and Osborne, 1972), increasing ethylene synthesis (Riov et al., 1990) and/or by induction of cellulases actively involved in abscission (Abeles, 1969).

Ethylene and Abscission

The plant hormone, ethylene, is a very simple gaseous molecule with a complex role in plant biology (Roman et al., 1995; Abeles et al., 1992). It is generally required in very small amounts to modify plant growth and development and has been shown to play a fundamental role in fruit ripening, seed germination, sex determination, leaf abscission, flower senescence, response to mechanical stress and also pathogenic responses (Abeles et al., 1992; Bleecker and Kende, 2000). Either the biosynthesis or the perception of the ethylene gas controls these effects (Roman et al., 1995; Bleecker and Kende, 2000). Ethylene can be produced by almost all parts of the plants, although the rate of production depends on the tissue type and the stage of development. Ethylene has been studied extensively in relation to abscission and senescence due to its detrimental effects on shelf life of plant products. Ethylene production increases during abscission as well as during stresses such as: drought, flooding, chilling and mechanical wounding. Stress ethylene may stimulate stress responses in plants such as abscission and senescence, including that of flowers (Taiz and Zieger, 1998; Van Doorn and Vojinovic, 1996). The promotion of abscission by ethylene occurs in almost all plant parts (Sexton, 1997). The shedding of leaves, fruits, petals, sepals, buds and stems is induced by ethylene in a wide variety of plants (Taiz and Zieger, 1998, Sexton, 1997). The response to ethylene depends to a great extent on the concentration and the duration of the exposure, as well as the plant organ and the levels of receptors (Høyer, 1996; Sexton, 1997). However, the responsiveness of the abscission zone to the ethylene varies between organs of the same plant (Aloni et al., 1994). Therefore, any changes in the response will be attributed to changes in receptor concentration or functioning of the following signal transduction pathway.

Ethylene + receptor → ethylene receptor complex → abscission

The increase in ethylene production and/or sensitivity are regarded as providing an inductive stimulus at the onset of floral abscission (Brown, 1997). However, not all researchers are convinced that this is the only factor/process involved (Sexton, 1997). According to some, auxin and ABA may also be primary regulators of the abscission process (Sexton, 1995; Kitsaki et al. 1999).

The involvement of ethylene in natural abscission has been extensively studied by demonstrating the increase in ethylene production rates prior to abscission (Sexton, 1997); however, showing causal relationship has been difficult (Brown, 1997). The levels of ethylene in the abscission zone have been shown to be higher during abscission (Patterson, 2001). There is also evidence that flowers and young fruit produce ethylene, and this ethylene has been associated with petal abscission (Pratt and Goeschi, 1969; Van Doorn, 2001). An adequate amount of ethylene must be produced long before abscission to cause a response (Lipe and Morgan, 1973). In a study of *H. rosa-sinensis*, Swanson et al. (1975), detected an increase in ethylene production in the abscission zone prior to and during abscission reaching a peak after abscission was completed. Ethylene production appears to accelerate and coordinate the abscission of raspberries (Burdon and Sexton, 1990), which is important to synchronize the separation process (Guinn et al., 1990). Ethylene production was found to increase as the tissue ages indicating that abscission and ethylene production occur in concert with senescence (Swanson et al., 1975). Petals isolated from *H. rosa-sinensis* flowers were found to increase in ethylene production as senescence approached (Woodson et al., 1985). Cotton flowers were found to produce sufficient amounts of ethylene before abscission to suggest that ethylene might be a regulator in natural abscission (Lipe and Morgan, 1972). However, Marynick (1977) did not find a positive correlation between ethylene and abscission in cotton flowers. When ethylene production does not correlate with abscission, changes in tissue responsiveness to ethylene are then thought to be an important factor (Guinn et al., 1990). Sensitivity to ethylene has been shown to change if the plant is under environmental stress, such as water stress, and therefore, the amount of ethylene produced during abscission may not necessary be produced due to abscission (Brown, 1997).

Pollination of flowers appears to play a role in ethylene mediated natural abscission. Pollination often accelerates ethylene production (Jones and Woodson, 1999a, 1999b, 1997; Tang and Woodson, 1996; Larsen et al., 1995; Dervinis et al., 2000). Sexton et al. (1985) reported that there was no clear correlation found between ethylene and abscission in the absence of pollination. According to Van Doorn (2002), flowers that lose color or form when pollinated also show similar symptoms when exposed to ethylene, and the development of the symptoms can be accelerated. Pollination of *Pelargonium* was found to induce ethylene production, which then induced premature wilting and abscission of the petals (Dervinis et al., 2000).

In many species, ethylene may play an important role in inter-organ signaling related to pollination responses (Stead, 1992; Larsen et al., 1993, 1995; Woltering et al., 1997). Supporting this theory is the fact that ethylene can substitute for pollination in the initiation and acceleration of changes in flowers. Therefore, endogenous ethylene levels and changes in sensitivity prior to abscission of flowers exhibiting increased ethylene synthesis during development and after pollination, are of major importance in regulation of abscission.

A more universal approach to studying the promotion of abscission by ethylene seems to be by exposing the abscission zone to exogenous ethylene. Ethylene accelerates abscission of leaves, fruits, petals and buds in a wide variety of plants (Sexton et al., 1984; Woltering and Van Doorn, 1988; Sexton, 1997; Patterson, 2001). Exogenous ethylene was found to stimulate abscission of young fruits and buds of *Gossypium hirsutum* (Hall et al., 1957). Flower petals of *Pelargonium* treated with exogenous ethylene either wilt or abscise prematurely (Dervinis et al., 2000). The response to external ethylene is usually consistent within families and high sensitivity to ethylene exposure coincides with a regulatory role of endogenous ethylene during abscission (Van Doorn, 2001).

The sensitivity of the abscission zone to applied ethylene may vary between species and also between different organs of the same plant (Sexton, 1997). Minimal concentrations of ethylene, between 0.1 and 1.0 μl^{-1}, appear to cause abscission (Sexton, 1997). In fruiting cotton plants, exposure to 0.5 $\mu l\ l^{-1}$ ethylene caused 100% abscission in young fruits and floral buds (Lipe and Morgan, 1973). Høyer (1985) found that exposing *Begonia-elatior* 'Sirene' to 0.05, 0.1, 1.0 and 5.0 $\mu l.\ l^{-1}$ ethylene for 72 hours caused flower abscission. Potted Kalanchoe have also been known for their sensitivity to ethylene, with concentrations as low as 0.5 $\mu l.l^{-1}$ causing flowers to inroll and abscise (Marousky and Harbaugh, 1979). The extent of ethylene effects on abscission depends on both concentration and duration of exposure. In *Begonia-elatior* 'Sirene' the reaction to ethylene was dependent on both concentration and exposure time and that the exposure time was more important in reducing the number of flowers than the concentration (Høyer, 1985). Kalanchoe petals required an exposure of about 8 hours before inrolling started and 24 hours to reach the minimum diameter (Serek and Reid, 2000).

The developmental stage of the organ at the time of exposure to ethylene is also an important factor to be considered when studying ethylene regulation of abscission. Even high concentrations of ethylene may not cause abscission at certain stages of development. The sensitivity of the tissue to ethylene appears to change with maturation (Evensen, 1991; Høyer, 1996) and several studies support this claim. In *Pelargonium x domesticum* florets, exposure to 100 $ml.l^{-1}$ of ethylene on freshly opened flowers did not cause abscission, whereas older flowers abscised when exposed to only 0.5 $ml.l^{-1}$ of ethylene (Evensen, 1991). Ethylene exposure induced senescence in mature petals of *Hibiscus* flowers following anthesis but not in petals taken from buds before anthesis (Woodson et al., 1985). Peach flowers have three abscission zones, each of which become sensitive to ethylene at a different developmental stage (Zanchin et al., 1995).

Abscission appears to be generally regulated by ethylene, but there are exceptions in some species (Van Doorn, 2002). In addition to the measurement of ethylene during abscission and exposure of the plant to ethylene, inhibitors of ethylene formation and action have been used to investigate the role of ethylene in abscission.

Ethylene Inhibitors

The role of ethylene in abscission have been studied using inhibitors of ethylene synthesis and action. Aminoethoxyvinyl glycine (AVG), which interferes with ethylene synthesis (acts as a block), has been shown to reduce the production of endogenous ethylene in many plant systems (Sexton, 1997). This reduction in ethylene biosynthesis effectively delays abscission. AVG has been used mostly as a research tool to identify the role of ethylene in abscission and has received very little attention commercially since it does not protect the plants from exogenous ethylene. Also another very important reason that it is not used more on ornamentals is that phytotoxic effects are common on whole plants (Barrett, personal communication). When cocoa flowers were treated with AVG abscission was delayed but not prevented (Aneja at al., 1999). In another study, Sagee et al. (1980) reported that AVG prevented ABA-induced abscission in citrus explants.

Similarly, silver thiosulfate (STS) was found to be effective in blocking ethylene synthesis (Macnish et al., 1999). STS is thought to be the most effective inhibitor of ethylene responses because it competes with copper localized within ethylene receptors (Cameron and Reid, 1982). Beyer (1979) showed that Ag^+ is capable of inhibiting ethylene metabolism as well as action. Once STS is applied as a spray, or pulsed in to the stem, it effectively blocks the downstream events that lead to senescence and abscission.

STS has proven to be an effective tool for controlling abscission as it has reduced ethylene-induced abscission in many species (Cameron and Reid, 1982).

STS was shown to successfully reduce or prevent abscission in Geraniums (Cameron and Reid, 1982), cut phlox flowers (Porat et al., 1995), cut carnation (Serek et al., 1995), potted Begonia (Serek et al, 1994b), miniature potted roses (Serek and Reid, 1993) and many others. STS is widely used commercially; however, even with its success in inhibiting abscission there are drawbacks to its use. One is that silver is a heavy metal pollutant and this has raised concerns about ground water pollution (Cameron and Reid, 2001). An additional drawback is that STS has a narrow concentration range at which it is effective in inhibiting ethylene action without causing phytotoxicity (Rewinkel-Jansen, 1986; Nell, 1993).

Several research projects have focused on STS and abscission in *H. rosa-sinensis*. Thaxton et al., (1988) studied the effect of STS on *H. rosa-sinensis* bud abscission during ethylene exposure and showed that spraying the plants with 4 mM STS was effective in reducing ethylene induced bud abscission. Woodson et al. (1985) found that STS treatment prevented senescence and extended life of *H. rosa-sinensis* petals excised from flowers the day before flower opening, while treatment on the morning of flower opening was not effective. Similarly, Reid et al. (2002) reported that treatment of open *H. rosa-sinensis* flowers with STS had only a modest effect in extending flower life. When they applied 1 μmol Ag^+ to the flower, its senescence was extended by one day. Others have reported that treatment with STS reduced abscission of *H. rosa-sinensis* induced by either darkness, temperature or ethylene (Rystedt, 1982; Høyer, 1984,1986; Thaxton et al., 1988).

1-Methylcyclopropene (1-MCP), invented by Sisler in the late 1980s (Blankenship, 2003), is a very effective blocking agent for ethylene receptors and can inhibit ethylene responses for extended durations (Serek et al., 1994a; Sisler et al., 1996). This inhibitor protects plant products from both endogenous and exogenous ethylene and prevents ethylene effects in a broad range of fruits, vegetables and ornamentals (Blankenship and Dole, 2003).

In numerous cut flower species and potted plants, 1-MCP was found to prevent damage caused by ethylene exposure (Blankenship and Dole, 2003), which includes: accelerated bud and flower abscission of oriental lily (Celikel at al., 2002); flower abscission of garden phlox (Porat et al., 1995); flower and petal abscission of carnation, alstroemeria, snapdragon, larkspur and sweet William (Serek et al., 1995a); Begonia flower abscission, senescence in Kalanchoe and bud abscission in rose (Serek et al., 1994b, 1995b).

In *H. rosa-sinensis* 1-MCP had only a modest effect on extending the life of the flower and only continuous treatment with 1-MCP delayed senescence (Reid et al., 2002). Similar results were shown in Begonia, as there was no increase in shelf life in an interior environment for plants treated with 1-MCP (Serek et al., 1994b). Cameron and Reid (2001) reported similar observations with geraniums, and they showed that geranium required multiple applications of 1-MCP to achieve complete protection to ethylene. These results showing a transient effect on some crops is probably because 1-MCP binds permanently to receptors present at the time of treatment and any new receptors formed after the treatment will be sensitive to ethylene (Blankenship and Dole, 2003). Supporting this theory, Newman et al. (1998) found that senescence of gypsophila flowers was delayed when treated with either 1-MCP or STS at the time of opening; however, senescence of the buds not open at the time of treatment was only effectively delayed by STS. STS appears to remain active in the plant tissue longer than 1-MCP allowing the plant to block any new receptors forming as the plant develops (Blankenship and Dole, 2003).

It has not yet been determined whether these inhibitors of ethylene just slow down abscission or stop it completely. The latter would be expected if ethylene alone mediated abscission. Much research is still needed to clarify the role of ethylene requirement for the induction of abscission. The availability of mutants as well as ethylene inhibitors provides better tools to study ethylene and its relationship to abscission.

CHAPTER 3
INTERACTIONS BETWEEN SHIPPING, TEMPERATURE, DURATION AND ETHYLENE IN FLOWER BUD ABSCISSION OF *H .rosa-sinensis*

Proper shipping temperature and duration are critical in maintaining the quality of flowering potted plants. Owing to the detrimental effects of shipping, considerable research has been conducted to evaluate the effects of shipping duration and temperature and the involvement of ethylene on flower and flower bud abscission of *H. rosa-sinensis* (Nell and Barrett, 1984; Force et al., 1988; Thaxton et al., 1988; Van Meeteren and Van Gelder, 1995, 2000; Høyer, 1996). Flower, flower bud and leaf abscission in potted *H. rosa-sinensis* were greater when shipping temperature was high, as there was approximately 10 times more bud abscission at 30° C compared to 10° C (Thaxton et al., 1988). The effect of shipping temperature was also shown in potted roses, as flower longevity decreased and leaf abscission increased when plants were shipped at 28° C for durations longer than 2 days and when shipped at 16° C for 4 days or longer compared to plants shipped at 4° C (Cushman et al., 1998).

In addition to high temperature, other factors such as darkness or low light may induce abscission (Decouteau and Craker, 1983). Shipping in darkness greatly reduced *H. rosa-sinensis* plant quality (Gibbs et al., 1989; Van Meeteren and Van Gelder, 1995). Duration of shipping has also been shown to have a profound effect on the plants (Poole and Conover, 1987; Nell and Barrett, 1984; Cushman et al., 1998). Nell and Barrett (1984) reported an increase in bud abscission on *H. rosa-sinensis* plants held at 5° C and

27° C for 4 days or longer compared to plants shipped at 16° C. They also found that detrimental effects of shipping at 5 and 27° C increased at longer shipping durations.

Abscission of *H. rosa-sinensis* buds and leaves in response to ethylene has been previously documented (Høyer, 1984, 1996; Thaxton et al., 1988). Høyer (1996) found *H. rosa-sinensis* very sensitive to exogenous ethylene and Van Doorn (2001) classified *H. rosa-sinensis* as class 4 in sensitivity to ethylene, which means 100 % response is seen by the end of 24 hrs exposure to a concentration of 3 $\mu L.l^{-1}$. Ethylene has been implicated in bud abscission of *H. rosa-sinensis* during shipping (Høyer, 1995). Supporting this claim are reports by Høyer (1986) and Thaxton et al. (1988) that silver thiosulphate (STS) can reduce low-light-induced bud abscission. During distribution and transport of Hibiscus, the plants may be exposed to exogenous ethylene. In addition, plants in shipping are thought to produce ethylene due to stress and darkness, causing the loss of buds and reduced plant quality (Thaxton et al., 1988). Other factors such as low irradiance or increase in sink demand have been shown to increase flower bud abscission (Force et al., 1988).

Previous research on *H. rosa-sinensis* contained conflicting results in regards to bud abscission. Force et al. (1988) reported that young buds were the most sensitive to ethylene exposure, while Rystedt (1982) indicated that they were the least sensitive. This contradiction may be due to differences in the definition of young buds. Van Meeteren and Van Gelder (1995, 2000) recorded data on flower buds with bud length between 5 and 10 mm, whereas Nell and Barrett (1984) divided the buds into two categories: those less than 0.5 inches (13 mm) and those greater than 0.5 inches. Gibbs et al. (1989) did not divide flower buds into different stages, and Høyer (1996) described the flower buds in more general terms: open flowers, green buds and buds showing color. Force et al. (1988) divided the buds into 3 categories, very young buds (< 10 mm), expanding buds (10-30 mm) and mature buds (> 30 mm). Thaxton et al. (1988) gave the most detailed description of developmental stages of flower buds of *H. rosa-sinensis.* They divided the buds into four developmental stages: stage 1 (> 4 cm length with corolla extended beyond calyx), stage 2 (3 – 4 cm with pigmented corolla visible), stage 3 (2 – 3 cm with no corolla visible) and stage 4 (< 2 cm with calyx not parted). However, their broader divisions between stages left wide gaps between developmental stages where differences in pattern of bud abscission may occur.

The role of ethylene in bud abscission of *H. rosa-sinensis* during shipping, and the pattern of bud drop has not been clearly addressed in previous studies. The following research was designed to establish the involvement of ethylene during shipping and the effect of shipping temperature and duration on the abscission pattern of flower buds at different stages of development.

Materials and Methods

Plant Material

H. rosa-sinensis 'Pink Versicolor' cuttings rooted in Oasis media, were obtained from a commercial nursery (Yoder Brothers, Parrish, FL). The cuttings were immediately planted into 10-cm (600-ml) plastic pots containing a commercial media (Fafard mix # 2, Conrad Fafard Inc, MA) and grown in a temperature controlled double polyethylene greenhouse maintained between 26-30° C day and 21-24° C night temperatures under prevailing natural days. Plants were fertigated by drip irrigation daily with 20 N – 4.4 P – 16.6 K with N concentration of 150 $mg.L^{-1}$ using Scotts Peters fertilizer (Scotts –Sierra Horticultural Products Co., OH). Two weeks after potting, the plants were pinched to approximately three nodes and then treated with 21 ml.L^{-1} [chlormequat chloride (2-chloroethyl) trimethylammonium chloride] (Cycocel) to obtain compact growth. The plants were allowed to flower freely under the greenhouse conditions with no further treatments to the plants. All open flowers were removed from each plant until a week before the beginning of an experiment and then subsequent flowers were allowed to develop and senesce naturally.

Flower Bud Tagging

In this study, flower bud development was divided more extensively than was done in previous reports in the literature to obtain a broader understanding of how the different stages responded to treatments. In all experiments, buds were grouped into six different developmental stages ranging from very young buds (stage 1) to the opening flower (stage 6) (Table 3-1). Stage 1 and 2 buds were measured by taking the length of the bud beginning at the bottom of the calyx to the top of the actual bud. At the beginning of each experiment, two flower buds of each stage were randomly chosen on each plant and tagged before applying treatments. This was considered to be day 0 of the experiment.

Experiment 1: Shipping Duration and Temperature Effect on Flower Bud Abscission

The plants were tagged and boxed on day 0, then placed in simulated shipping for 2, 4 or 6 days. Three temperature treatments were imposed during the simulated shipping (13, 18 or 25° C). A control treatment, consisting of tagged plants kept in the greenhouse for the duration of the experiment, was used as a basis for comparison of the effect of shipping on bud abscission. At the end of the shipping period, the plants were unboxed

Table 3-1. Developmental stages of *H. rosa-sinensis* floral buds

Bud Stages	Description:
Stage 1	Very young bud with calyx not parted - < 1 cm in length.
Stage 2	Calyx is slightly open and bud is between 1 and 2 cm in length.
Stage 3	Expanding bud with calyx completely open but no corolla visible.
Stage 4	Pigmented corolla is just visible, but less than ¼ of corolla is showing.
Stage 5	More than ¼ of the corolla is visible, but the corolla is still rolled tight.
Stage 6	Open flower.

and placed back in the greenhouse. The number of remaining tagged buds was recorded on day 0, on the day of removal from shipping and then every other day through day 14. This experiment was conducted during July 2001 following preliminary experiments with shipping durations.

The experiment was set up in a RCBD with 3 blocks. For each treatment there were 3 plants per block tagged as described previously, giving a total of 6 buds of each stage in a block for each treatment. Statistical analysis of the data was performed using SAS, version 6.12 (SAS Institute Inc., Cary, NC). Tukey's W Procedure at alpha = 0.05 was used for mean comparison.

Experiment 2: Ethylene Effect on Flower Bud Abscission

This experiment was performed to determine the sensitivity to ethylene of flower buds of *H. rosa-sinensis* 'Pink Versicolor' at the different developmental stages. The tagged plants were then treated with three ethylene concentrations (0, 1, or 3 $\mu L.l^{-1}$) for 24 hrs in the dark at 20° C. Treatment occurred in airtight glass aquariums where ethylene was injected into the chamber exposing the plants and flower buds to the different concentrations. Ethylene levels were checked before the end of the treatment by extracting gas samples from the chambers and using gas chromatography (Hewlett Packard 5890 Series II). Control plants were stored under identical conditions except they were injected with air instead of ethylene. After treatment, the plants were removed from the chambers and placed in a greenhouse for observation. The number of buds remaining per plant was recorded daily starting from treatment initiation (day 0) to day 8. The results are expressed as the number of buds retained on the plant over the time course of observation.

This experiment was conducted in the June 2001 and repeated again in October 2001. Five plants were tagged as described above giving a total of 10 buds of each developmental stage per block. A randomized complete block design (RCBD) with 3 blocks was used. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC) and analyzed using analysis of variance and means with standard error.

Experiment 3: The Effect of Low Ethylene Concentration on Abscission

Ten plants with all developmental bud stages (1 – 6) present were treated with ethylene at 0.2-0.3 $\mu L.l^{-1}$ for 16 or 24 hrs at 20° C. The plants were treated in airtight glass aquariums with measured amounts of ethylene injected into the chamber using a flow through method, which mixes air and ethylene before reaching the chamber. Control plants were placed in identical chambers with only air flowing through and potassium permanganate to extract ethylene that might have been produced by the plant during the treatment. Following the ethylene treatment the plants were placed in the greenhouse for observation. The greenhouse was maintained at 30/24° C (day/night). The observation of ethylene effects included yellowing of the flower buds as well as discoloration of the abscission zone. The experiment was conducted three times between September 23rd and October 3rd, 2003.

Results and Discussion

The problem of flower bud loss in *H. rosa-sinensis* has been reported previously in plants exposed to ethylene (Høyer, 1996) as well as in plants during dark shipping (Nell and Barrett, 1984).

Experiment 1: Shipping Duration and Temperature Effect on Flower Bud Abscission

Results on control plants showed that flower buds developed normally through flowering and senescence (Fig. 3-1). Stage 6 buds (open flower) senesced and dropped by day 2 followed by stage 5 buds dropping by day 4 after opening and senescing. On day 5, stage 4 buds developed into open flowers and began to senesce and similarly stage 3 buds started to drop on day 6. By the end of the observation period (day 14) approximately 80% of stage 2 buds had senesced and dropped while stage 1 buds continued to develop normally.

On temperature and shipping treatments, a comparison at day 6 showed a three-way interaction between shipping, temperature and bud stage ($Pr < .0001$) (Table 3-2 A). Comparisons between treatments were made by the Tukey's W Procedure at alpha = 0.05. Flower buds of all the stages on plants shipped at 13° C continued to develop normally through flowering and senescence. Bud drop on plants shipped at 13° C was similar to control plants however the rate of bud drop was slower probably due to the cooler temperature. Higher temperatures enhanced flower bud abscission, and the impact was greater with longer duration (4 and 6 days) of shipping. Shorter duration of shipping

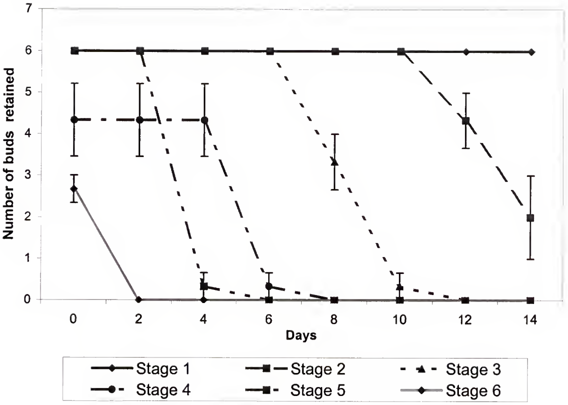

Figure 3-1. Total number of flower buds at six developmental stages retained per replicate plants maintained in a greenhouse at 30/24° C (day/night). Day 0 is the day the plants were tagged.

(2 days) had no apparent effect on bud drop even at high temperatures (Tables 3-2 to 3-5). Higher temperature (25° C) and increased shipping time (6 days) caused markedly greater premature bud drop over time for the younger stages (stages 1 and 2) compared to older stages (3, 4 and 5) (Table 3-3 and 3-4). Stage 1 buds showed a constant decrease in number of buds retained when plants were shipped at 25° C for 6 days where on day 6 the number of buds retained was 4.7 dropping to 1 by day 12. These results are in agreement with Nell and Barrett (1984) as they reported that smaller buds (less than 0.5 inches- considered as stage 2 in the present study) abscised 2 to 4 times more than buds greater than 0.5 inches during dark shipping at high temperature. They also reported that most of

Table 3-2. Number of buds retained on day 6 in greenhouse for the three shipping durations (2, 4 and 6 days) and three shipping temperatures (13, 18 and 25° C)

	Stages						
Shipping duration	Temperature °C	1	2	3	4	5	6
2 days	13	6	6	6	4.7	1	0
	18	6	6	6	5.7	0.3	0
	25	6	6	5.3	4	0	0
4 days	13	6	6	6	5.3	0.3	0
	18	6	6	6	5.3	0.3	0
	25	5.7	6	6	3.3	0	0
6 days	13	6	6	5	5.3	0.3	0
	18	6	6	5.7	4	0	0
	25	4.7	5.7	5.3	2	0	0
Factorial Pr > F							
Shipping	0.6221^{ns}						
Temperature	$< .0001^{***}$						
Ship*Temp	$< .0001^{***}$						
Stage	$< .0001^{***}$						
Shipping*Stage	$< .0001^{***}$						
Temperature*Stage	$< .0001^{***}$						
Ship*Temp*Stage	$< .0001^{***}$						
$HSD_{0.05}$	1.9						

$HSD_{0.05}$ = Honest Significant Difference at 0.05 Probability
ns: not significant, * significant at 0.05, *** significant at 0.0001

Table 3-3. Number of buds retained on day 8 in greenhouse for the three shipping durations (2, 4 and 6 days) and three shipping temperatures (13, 18 and 25° C)

	Stages						
Shipping duration	Temperature °C	1	2	3	4	5	6
2 days	13	6	6	5.7	2.3	0.3	0
	18	6	6	5.3	0.3	0	0
	25	6	6	3.7	0	0	0
4 days	13	6	6	6	5	0.3	0
	18	5.7	6	5.3	2.3	0	0
	25	4.7	4.3	5.3	0.3	0	0
6 days	13	6	6	5	5.3	0.3	0
	18	5.7	6	5.3	1.3	0	0
	25	2.3	2	4	0	0	0
Factorial Pr > F							
Shipping	0.0748^{ns}						
Temperature	$< 0.0001^{***}$						
Ship*Temp	$< 0.0002^{*}$						
Stage	$< 0.0001^{***}$						
Shipping*Stage	$< 0.0001^{***}$						
Temperature*Stage	$< 0.0001^{***}$						
Ship*Temp*Stage	$< 0.0001^{*}$						
$HSD_{0.05}$	2.9						

$HSD_{0.05}$ = Honest Significant Difference at 0.05 Probability

ns: not significant, * significant at 0.05, *** significant at 0.0001

Table 3-4. Number of buds retained on day 10 in greenhouse for the three shipping durations (2, 4 and 6 days) and three shipping temperatures (13, 18 and 25° C)

	Stages						
Shipping duration	Temperature °C	1	2	3	4	5	6
2 days	13	6	6	1.2	0	0	0
	18	6	6	2	0	0	0
	25	6	6	0.3	0	0	0
4 days	13	6	6	6	0	0	0
	18	5.7	6	3.7	0	0	0
	25	4	4	0	0	0	0
6 days	13	6	6	4.3	4.7	0	0
	18	5.3	6	5.3	0.3	0	0
	25	2.3	1.7	1.7	0	0	0
Factorial Pr > F							
Shipping	0.3075^{ns}						
Temperature	$< .0001^{***}$						
Ship*Temp	$< .0001^{***}$						
Stage	$< .0001^{***}$						
Shipping*Stage	$< .0001^{***}$						
Temperature*Stage	$< .0001^{***}$						
Ship*Temp*Stage	$< .0001^{***}$						
$HSD_{0.05}$	2.3						

$HSD_{0.05}$ = Honest Significant Difference at 0.05 Probability

ns: not significant, * significant at 0.05, *** significant at 0.0001

Table 3-5. Number of buds retained on day 12 in greenhouse for the three shipping durations (2, 4 and 6 days) and three shipping temperatures (13, 18 and 25° C)

	Stages						
Shipping duration	Temperature °C	1	2	3	4	5	6
2 days	13	6	6	0	0	0	0
	18	6	5.7	0.3	0	0	0
	25	6	4	0	0	0	0
4 days	13	6	6	2	0	0	0
	18	5.7	6	1.3	0	0	0
	25	4	3.3	0	0	0	0
6 days	13	6	6	4.3	0	0	0
	18	5.3	6	2	0	0	0
	25	1	1.3	0	0	0	0
Factorial Pr > F							
Shipping	0.4813^{ns}						
Temperature	$< .0001^{***}$						
Ship*Temp	$< .0001^{***}$						
Stage	$< .0001^{***}$						
Shipping*Stage	$< .0001^{***}$						
Temperature*Stage	$< .0001^{***}$						
Ship*Temp*Stage	$< .0001^{***}$						
$HSD_{0.05}$	1.8						

$HSD_{0.05}$ = Honest Significant Difference at 0.05 Probability
ns: not significant, * significant at 0.05, *** significant at 0.0001

the buds abscised 5 days following the shipping treatment on plants held at 27° C regardless of duration of shipping (2, 4 or 7 days). Results obtained in the present experiment do not agree with Nell and Barrett (1984) because shipping for 2 days at 25° C did not cause premature flower bud abscission. On day 6 all stage 1 and 2 buds were retained but over time and in longer shipping (4 and 6 days) and 25° C considerable abscission was recorded. Thaxton et al. (1988) also reported that shipping for four days at 30° C caused 10-fold greater bud abscission than 10° C indicating the effect of high temperature on abscission however they did not specify which bud stages were more affected. Thaxton et al. (1988) also reported that plants shipped at 30° C were considerably more chlorotic, which was not observed in the present experiment. No visible effect was observed on the leaves during shipping even at 25° C and for 6 days.

The number of buds abscised increased during the postshipping observation, and by Day 14 all the buds from different stages had abscised on plants held at 25° C for 6 days (data not shown). Similarly, Force et al. (1988) found that the percentage of bud abscission increased during the postshipping period, recording 93 % of the buds abscised 10 days after the dark treatment. Stage 3 – 6 did not abscise in response to shipping duration and often opened and senesced normally during and post-shipping treatment. These results are in agreement with earlier work by Force et al. (1988), who also reported that mature buds were more likely to open and abscise during and after dark storage treatment.

Stages 1 and 2 exhibited high rates of abscission under higher temperature treatments and longer durations postshipping. In a preliminary experiment, plants were shipped at 25° C for 2, 4 and 6 days. A significant difference was found between the shipping durations with longer shipping 4 and 6 days causing extensive bud abscission compared to 2 days (data not shown). It was expected that bud abscission of *H. rosa-sinensis* would result from high temperature during shipping as it has been previously reported by others (Gibbs et al., 1989; Thaxton et al., 1988). Shipping for longer durations was found to exhibit more damaging effects on *H. rosa-sinensis* compared to shorter durations (Nell and Barrett, 1984). They found that shipping for 7 days at either low (5° C) or high (27° C) temperatures caused bud drop.

Experiment 2: Ethylene Effect on Flower Bud Abscission

Figure 3-2 and 3-3 shows the number of flower buds retained by the plants during the observation period starting on the day of ethylene treatment to day 8. The ethylene treatment accelerated abscission of *H. rosa-sinensis* buds at all stages of development (<0.0001) (Figure 3-3) compared to control where all the flower buds develop normally through flowering and senescence (Figure 3-2). No significant difference was found between the 1 and 3 $\mu L.l^{-1}$ ethylene concentrations. The comparisons were made by means with standard error via SAS (version 8.2). The results showed that buds showing color (Stages 5) were the first to start abscission in response to ethylene and more than 50% dropped during the 24 hrs exposure and 100% dropped by day 2. Similarly stage 4 buds began to abscise soon after the removal from ethylene exposure but 100 % of the buds abscised by day 3. The remaining buds showed a slower beginning to abscission. Stage 3 buds started to drop on day 3 and by day 4 all the buds were lost followed by stage 2 buds, which began to drop their buds excessively by day 3 dropping all their buds by day 5. Finally stage 1 buds started to drop on day 4 and by day 8 only 40 % of the buds were retained and these buds continued to develop normally. These buds did not go through the normal development and they abscised prematurely without further development occurring.

The plants treated with ethylene had a pronounced discoloration (white) and swelling of the calyx abscission zone (found 1.5 cm beneath the calyx) (Figure 3-4) compared to the normal brown/red coloration of control plants (Figure 3-4). It has been previously documented that *H. rosa-sinensis* is sensitive to ethylene and that exposure to ethylene would cause bud and leaf abscission (Van Doorn, 2001; Høyer, 1996; Thaxton et al., 1988; Woltering, 1987). In this experiment, the larger buds (Stage 6 and 5) dropped first in response to ethylene followed by the smaller buds (Stage 4, 3, 2 and finally 1) agreeing with the earlier results of Høyer (1996). No yellowing or leaf

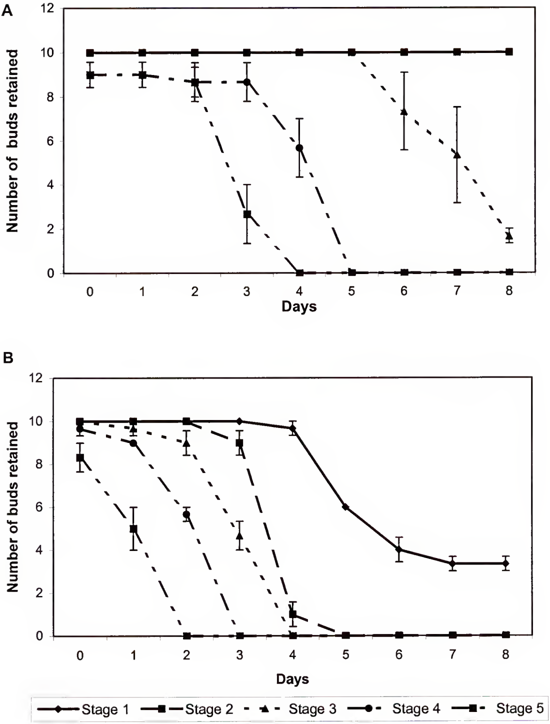

Figure 3-2 and 3-3. Total numbers of flower buds at five developmental stages retained per replicate plants maintained in a greenhouse at 30/24° C (day/night) then exposed to A. 0 µL l^{-1}; B. 1 µL.l^{-1} ethylene for 24 hrs respectively. Day 0 is the day tagged plants are placed in treatments; day 1 the plants are removed from treatment and kept in greenhouse for the duration of the experiment

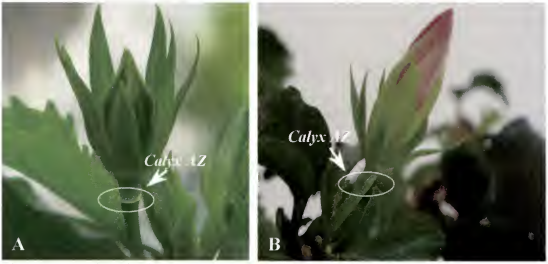

Figure 3-4. Calyx abscission zone of A. Stage 3 bud showing swelling and discoloration following 24 hrs of ethylene treatment at 1 $\mu L.l^{-1}$ and B. Stage 5 bud on control plant showing normal brown/red coloration surrounding the abscission zone

abscission was detected during the course of this study, unlike Høyer (1996) who reported that yellowing of the leaves appeared soon after the exposure to ethylene and leaf abscission occurred 8 days later. In general, there is a gradual increase in sensitivity of flowers to ethylene with increasing age (Evensen, 1991; Woodsen et al., 1985).

The pattern of bud drop in this experiment did not agree with the bud drop during shipping (experiment 1). During shipping abscission was only on Stage 1 and 2 buds while Stage 3 and larger buds continued to develop normally whereas when the plants were exposed to 1 $\mu L.l^{-1}$ the larger buds (Stage 5) dropped first followed by the smaller buds. Due to the differences observed in the pattern of bud drop experiment 3 was conducted to try and simulate the concentration of ethylene produced during shipping.

Experiment 3: Low Ethylene Concentration Effect on Abscission

There were significantly more buds on control plants compared to plants treated with low ethylene concentrations (0.2-0.3 $\mu L.l^{-1}$). There was a pronounced difference by day 5 between 16 and 24 hrs of ethylene exposure. The 24-hour ethylene exposure caused most of the flower buds (90 %) to abscise within 5 days, with larger buds (Stages 3-6) dropping first followed by smaller buds (Stages 1 and 2). Yellowing of the bud and discoloration of calyx abscission zone was noticed after the ethylene treatment, stopping any further development of the buds. Both symptoms were typical of ethylene damage on the flower buds as observed in preliminary experiments and experiment 2. There was no visible effect on any of the bud stages on the plants for the first 4 days following the ethylene treatment for 16 hours. On day 5, a few of the Stage 1 and 2 buds began to yellow however, approximately 75 % abscised by day 7 (Fig. 3-5). These results are in disagreement with the work of Høyer (1996) who reported that abscission of buds showing color (Stage 4 and larger) started after an ethylene exposure of 0.1 $\mu L.l^{-1}$ for 12 hrs compared to green buds (Stage 3 and smaller) that abscised after 1.0 $\mu L.l^{-1}$ for 24 hrs. In this study 16 hrs of low ethylene treatment did not affect Stage 3 and larger buds as they continued to develop normally however Høyer (1996) reported that the total abscission of buds showing color at lower ethylene concentration was higher compared to green buds.

From these three experiments it can be seen that flower bud abscission of *H. rosa-sinensis* 'Pink Versicolor' is strongly affected by shipping temperature and duration. High shipping temperature (25°C) and longer durations (4 and 6 days) causes significant loss of buds during and after shipping. Exposure of *H. rosa-sinensis* to ethylene resulted in significant bud loss of all bud stages compared to control plants. From the present study, it is clear that the pattern of flower bud drop during shipping was not found to be

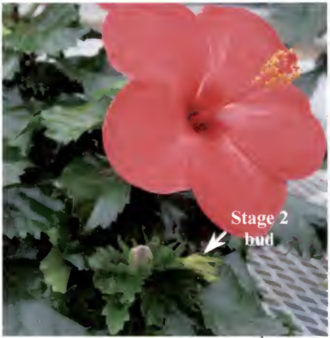

Figure 3-5. *H. rosa-sinensis* plant treated with 0.2-0.3 μL l^{-1} ethylene for 16 hours showing yellowing and abscising stage 1 and 2 buds while the larger buds developing normally. Arrow showing an abscised yellow Stage 2 buds

parallel to flower bud drop on plants exposed to ethylene. During shipping the smaller buds (Stage 1 and 2) appear to be affected by the shipping conditions whereas the larger buds (Stage 3 and above) develop normally until they senesce. In contrast, exposure to ethylene cause the flower buds to prematurely abscise with the larger buds (Stage 6) dropping first followed by the smaller buds.

Dividing the buds into six stages provided more information on the exact developmental stage affected during shipping as well as cleared the contradictions found in the literature. According to Force et al. (1988), buds less than 10 mm or larger than 30 mm were less sensitive to low light or darkness compared to buds in the size of 10-30 mm. Høyer (1996) reported that abscission occurred in both green buds and buds showing color when plants were treated with darkness. Both studies disagree with the finding in this present experiment. Results obtained from the present study clearly show that during shipping Stage 1 and 2 are the most affected while ethylene accelerate bud abscission of all stages but the larger buds are more sensitive. The sensitivity found in this study confirms the sensitivity found by Woltering (1987).

The relationship between dark shipping or storage and ethylene of *H. rosa-sinensis* has been previously studied (Høyer, 1986; Thaxton et al., 1988; Van Meeteren and Van Gelder, 1995). Ethylene has been implicated to cause the bud abscission during shipping however showing that ethylene is the only factor in induction of abscission has proven to be more difficult. The results in this study are in agreement with previous studies indicating that ethylene plays a major role in flower bud abscission. The difference in the pattern of bud drop between shipping and exposure to ethylene may be attributed to ethylene concentration. It appears that during shipping the plants produce very small amounts of ethylene, which only affect the smaller buds while allowing the larger buds to develop normally. This was proven in experiment 3 where low ethylene concentration (0.2-0.3 $\mu L.l^{-1}$) for 16 hours caused bud abscission of Stages 1 and 2 buds. In contradiction, Høyer (1996) reported that exposure of plants to 0.1 $\mu L.l^{-1}$ for 12 hrs caused colored buds to abscise compared to the green buds. Exposing the plant to exogenous ethylene and the use of ethylene blocks has previously provided useful information about the involvement of ethylene in abscission. Further study on the use of different ethylene blockers during shipping would help clarify the involvement of ethylene in bud abscission.

CHAPTER 4
THE USE OF ETHYLENE BLOCKERS DURING SHIPPING OF *H. rosa-sinensis* PLANTS

Ethylene plays a role in abscission of many plant parts in plant species. High sensitivity to exogenous ethylene has been found to coincide with the regulatory role of endogenous ethylene (Van Doorn, 2001). Compounds inhibiting ethylene action and/or synthesis can be useful in the study of this hormone (Veen, 1983). Variable results have been obtained with use of ethylene blockers depending on the responsiveness of the organ to ethylene. Silver thiosulphate (STS) was found to reduce ethylene induced abscission in several species (Cameron and Reid, 1981, 1983; Cameron et al., 1981) as well as delay senescence of ethylene sensitive flowers (Veen, 1983). Pretreatment of carnation with STS increased its longevity (Reid et al., 1980a) and it completely blocked the ethylene surge preceding wilting of the petals (Veen, 1979a). Silver thiosulphate also reduced abscission caused by stresses such as temperature and darkness (Cameron and Reid, 1983; Thaxton et al., 1988). Høyer (1986) showed that spraying *H. rosa-sinensis* plants with 0.45 or 4.05 mM STS 11 days before dark treatment reduced bud drop compared to plants kept in dark without STS. However, high concentrations of STS caused injury to the flowers (Høyer, 1986).

With the concerns regarding the heavy metal pollution caused by STS, 1-Methylcyclopropene (1-MCP) has proven to be a useful tool and a potential replacement for STS. 1-MCP has added one more option for extending the shelf life and quality of plants (Blankenship and Dole, 2003). In the presence of exogenous ethylene, 1-MCP protected many potted plants from damage caused by ethylene (Blankenship and Dole, 2003). However, it appears that in the absence of exogenously applied ethylene, 1-MCP has no effect on the normal senescence in some plants (Celikel et al., 2002; Elgar et al., 1999; Serek and Reid, 2000; Serek and Sisler, 2001). With *H. rosa-sinensis*, 1-MCP was not very effective in reducing natural senescence in the absence of applied ethylene (Blankenship and Dole, 2003). 1-MCP had no effect on delaying the senescence of open *H. rosa-sinensis* flowers unless flowers were held in 1-MCP continuously in the absence of ethylene (Reid et al., 2002). The variability in results reported among the different plants was evident and this may be due to new binding sites forming after 1- MCP treatment (Blankenship and Dole, 2003). Blankenship and Dole (2003) reported the new binding site theory where some plants regenerate new sites quickly while others take longer rendering the ineffectiveness seen by some plant species. In addition to species, growing environment and maturity of the organ treated may influence the result achieved by 1-MCP treatment (Blankenship and Dole, 2003).

Compared to other ethylene inhibitors, aminoethoxyvinyl glycine (AVG) has received the least amount of attention commercially because it does not protect the plant from exogenous ethylene effects. In previous studies, AVG has been shown to effectively reduce ethylene synthesis and consequently delay senescence and abscission. Aneja et al. (1999) showed that AVG prevented the production of ethylene in flowers of cocoa: however, abscission still occurred after 72 hours. AVG effectively prevented the abscission in buds of Christmas cactus in the absence of exogenous ethylene (Serek and Reid, 1993). Ethylene production in cut Cymbidium was drastically reduced when treated with AVG compared to untreated flowers, which resulted in increasing the vase life (Kwack et al., 1996).

The objective of the present study was to determine the role of ethylene during shipping of *H. rosa-sinensis* and to evaluate the effectiveness of ethylene inhibitors on abscission of the different developmental flower bud stages. The use of ethylene biosynthetic and action inhibitors allowed us to further our knowledge of the involvement of ethylene in flower bud abscission of *H. rosa-sinensis*.

Materials and Methods

Plant Material

Rooted cuttings of *H. rosa-sinensis* 'Pink Versicolor' in Oasis media, were obtained from a commercial nursery (Yoder Brothers, Parrish, FL) and immediately transplanted into 10-cm (600 ml) plastic pots containing a commercial media (Fafard mix # 2, Conrad Fafard Inc, MA). After transplanting the plants were transferred to a temperature controlled double polyethylene greenhouse maintained between 26-30° C day and 21-24° C night temperatures under natural photoperiod. Plants were fertigated daily via drip irrigation with 20 N – 4.4 P – 16.6 K with N concentration of 150 $mg.L^{-1}$ (Scott's Peters fertilizer, Scott's –Sierra Horticultural Products Co., OH). Two weeks after potting, the plants were pinched to approximately three nodes and then treated with 21 $ml.L^{-1}$ [chlormequat chloride (2-chloroethyl) trimethylammonium chloride] (Cycocel), to obtain compact growth. The plants were allowed to flower freely under greenhouse conditions with no further treatments. All open flowers were removed daily from each plant until a week before the beginning of the experiment and then subsequent flowers were allowed to develop naturally.

In this study, the flower bud development was divided into six different developmental stages ranging from very young buds (Stage 1) to the opening flower (Stage 6) (Table 4-1). At the beginning of each experiment, two flower buds of each stage were randomly chosen on each plant and tagged before applying treatments. This was considered to be day 0 of the experiment.

Table 4-1. Developmental stages of *H. rosa-sinensis* floral buds

Bud Stages	Description
Stage 1	Very young bud with calyx not parted - < 1 cm in length.
Stage 2	Calyx is slightly open and bud is between 1 and 2 cm in length.
Stage 3	Expanding bud with calyx completely open but no corolla visible.
Stage 4	Pigmented corolla is just visible, but less than ¼ of corolla is showing.
Stage 5	More than ¼ of the corolla is visible, but the corolla is still rolled tight.
Stage 6	Open flower.

Treatment Techniques

A series of experiments were conducted to study the effect of different ethylene blockers (1-MCP, AVG and STS) on inhibition of *H. rosa-sinensis* bud abscission during shipping and when exposed to ethylene with the following pretreatment techniques:

1. **1-MCP Treatments**: 1-MCP (as EthylBloc) was obtained from BioTechnologies for Horticulture, Inc., SC. Tagged plants were sealed in a 900000 cm^3 (900 liter) airtight Plexiglas chamber for treatments. Fifty grams of EthylBlock powder (formulated as a gas embedded in a cyclodextran polymer) was weighed and placed in a 50 ml glass beaker next to the inside wall of the chamber. The chamber was sealed before adding 10 ml of buffer through a hole on the lid since a significant percentage of 1-MCP is released immediately after addition of liquid. In all the experiments, 1-MCP was used at commercially recommended rate of 900 ppb treated for 6 hours at 20° C.

2. **AVG Treatment**: Tagged plants were sprayed with 178 mg $AVG.L^{-1}$ of distilled water (Abbott Laboratories, IL). The plants were allowed to air dry before being boxed.

3. **STS Treatment**: Tagged plants were sprayed with 3.12 ml STS.L^{-1} water (Biochemical Sciences Inc, NJ) then allowed to air dry before boxing.

Experiment 1: Ethylene Effect on 1-MCP Pretreated Plants.

The experiment was performed in the period from November 5 to 12, 2001 with the following treatments imposed on tagged plants:

4. 1-MCP treatment of plants followed by exposure to ethylene at 1 μL.l^{-1} for 24 hour.
5. Plants were treated with 1-MCP but not exposed to ethylene.
6. Plants were exposed to 1 μL.l^{-1} ethylene without treatment with 1-MCP.
7. Control plants (no 1-MCP, no ethylene) were placed in a chamber with air only for 24 hours

Following treatments all plants were placed in the greenhouse maintained at 30/24° C (Day/Night) and prevailing daylight for 8 days in order to observe the long-term effect of the treatments. During this period the number of buds retained on the plants was recorded. The number of buds remaining was found by applying a slight pressure on each tagged bud every day for the duration of the observation period beginning on day 1 following the treatments. The experiment was arranged in a randomized complete block design (RCBD) consisting of three replications containing five plants each. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC) and analyzed using analysis of variance and means with standard error.

Experiment 2: Duration of 1-MCP Effects

This experiment was conducted to determine the transient effect of 1-MCP over time. All the plants were tagged as previously described before imposing the following treatments:

1. 1-MCP treatment for 6 hours then immediately exposing plants to ethylene at 1 μL.l^{-1} for 24 hours.

2. 1-MCP treatment for 6 hours then plants were placed in the greenhouse for 2 days before exposing to ethylene at 1 $\mu L.l^{-1}$ for 24 hours.
3. 1-MCP treatment for 6 hours then plants were placed in the greenhouse for 4 days before exposing to same 1 $\mu L.l^{-1}$ ethylene for 24 hours.
4. 1-MCP treatment for 6 hours then plants were placed in the greenhouse with out further treatment.
5. Control plants were kept in the greenhouse with no 1-MCP and no ethylene.

After treating the plants with 1-MCP as described above the plants were returned to the greenhouse. At the various days (0, 2 and 4) after 1-MCP treatment plants were exposed to ethylene at 1 $\mu L.l^{-1}$ for 24 hours then placed back in the greenhouse for 10 days. Treatments 4 and 5 remained in the greenhouse for the duration of observation. During the period of observation the number of buds retained on the plants were recorded beginning on day 0. The experiment was arranged as a randomized complete block design (RCBD) consisting of three blocks with each block containing five plants. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC). Tukey's W Procedure at alpha = 0.05 was used for mean comparison. This experiment was carried out in January 2002.

Experiment 3: The Use of Ethylene Blockers in Shipping

Ethylene has been implicated to cause bud abscission during shipping. In order to fully investigate the response of *H. rosa-sinensis* to shipping as well as to determine ethylene involvement in bud abscission, this experiment was designed to test three widely used ethylene blocks. This experiment was conducted in March 2002 and again in July 2002.

Greenhouse-grown plants were tagged as previously described and given these treatments:

1. Plants pretreated with AVG were boxed and placed in shipping.
2. Plants pretreated with STS were boxed and placed in shipping.
3. Plants pretreated with 1-MCP were boxed and placed in shipping.
4. Control plants were boxed without any prior treatment.

Following treatment applications the boxed plants were placed in dark simulated shipping environment at a temperature of 25° C for 4 or 6 days. Upon removal from shipping the number of buds retained by the plants was determined before placing the plants in the greenhouse for an additional observation period until Day 12. During the observation period the plants were fertigated daily as described in the plant material section. The number of buds retained by the plants was recorded on each day following removal from the shipping. Each treatment consisted of five plants and three blocks arranged as RCBD. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC). The experiment was analyzed using analysis of variance (ANOVA) and means with standard error.

Experiment 4: 1-MCP and Shipping

1-MCP pretreated greenhouse grown plants were boxed and placed in dark simulated shipping at 25° C. The plants were placed in shipping for 2, 4 or 6 days, and then they were placed in the greenhouse for observation where they were fertigated daily. A control treatment was also applied where the plants were shipped after 6 hours of exposure to air in the airtight Plexiglas chamber. At removal from shipping and for 12 days the number of buds retained by the plants was recorded. The experiment, which was carried out during October 2001, was arranged as RCBD with three block and five plants per block. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC). Tukey's W Procedure at alpha = 0.05 was used for mean comparison.

Experiment 5: STS and Shipping

Tagged plants pretreated with STS were boxed and placed in dark simulated shipping at 20° C. The treated plants in addition to control plants, which were not treated, were shipped for 4 and 6 days. After removal from shipping the plants were placed in the greenhouse for observation and fertigated daily. The number of buds retained on the plants was recorded on Day 0 (beginning of shipping treatment), the day of removal from shipping (Day 4 or 6) and every day there after for 12 days. The experiment was arranged in a RCBD with three blocks and four plants per block. The data recorded were the summation of total number of buds retained by the plants. The data were analyzed using analysis of variance (ANOVA) and standard errors were calculated. The analysis was carried out using SAS, version 8.2 (SAS Institute Inc., Cary, NC). This experiment was conducted during October 2003.

Experiment 6: AVG and Shipping

This experiment was conducted during June 2003 to determine the effect of AVG on flower bud abscission during shipping. Plants were tagged and treated with AVG, then boxed and placed in a dark shipping environment set at two temperature treatments of 20 or 25° C. The plants were kept in the shipping environment for 3 or 6 days. Controls were also included where the plants were boxed and shipped with no prior AVG treatment. At the end of shipping duration, the plants were unboxed and placed in the greenhouse where they were fertigated daily for the 12-day observation period. The number of buds retained on the shipped plants was recorded on the day of treatment imposition (Day 0), the day of removal from shipping (Day 4 and 6) and every day thereafter. The experiment was arranged in a RCBD with three blocks and four plants per block. The experiment was set as a 3-way factorial with 2 rates (0 and 178 mg AVG.L^{-1}) x 2 Temperatures x 2 durations. Statistical analysis of the data was performed using SAS, version 8.2 (SAS Institute Inc., Cary, NC). The sum of buds retained was analyzed using analysis of variance (ANOVA) and means with standard error.

Results and Discussion

Knowing the response of *H. rosa-sinensis* to shipping and their sensitivity to ethylene, it was of an interest to find a method of shipping that would reduce flower bud abscission caused by ethylene. Ethylene blocks have proven to be a useful tool in studying the involvement of ethylene on abscission. Commercially, STS have been used with variable success during shipping of *H. rosa-sinensis* (Barrett, personal communication). In addition there are concerns about heavy metal pollution with the usage of silver. Silver thiosulphate has recently been classified as a plant growth regulator by the United States Environmental Protection Agency (EPA) but is has not been registered for use therefore it cannot be legally marketed (Cameron and Reid, 2001). The discovery of 1-MCP as a powerful environmentally friendly inhibitor of ethylene action added a new horticultural tool that can reduce the damage caused by ethylene (Prange and DeLong, 2003). 1-MCP is proposed to be an alternative to STS (Cameron and Reid, 2001). Aminoethoxyvinyl glycine (AVG), an inhibitor of ethylene biosynthesis has been mainly used as a studying tool and has not received a lot of attention commercially since it does not protect the plants from exogenous ethylene. In the present study, 1-MCP, STS and AVG were used to study the effect of dark shipping on bud abscission and further clarify the involvement of ethylene. Analysis of variance was applied in all the experiments to determine the significance of the factorial followed by reporting the means with standard error.

Experiment 1: Ethylene Effect on 1-MCP Pretreated Plants

A highly significant difference was found between the chemical treatments x bud stages x observation time ($Pr < 0.0001$). Comparisons were made by means with standard error via SAS 8.2 to obtain the differences between individual treatments. Stage1 flower buds abscised extensively by day 6 when exposed to ethylene compared to plants pretreated with 1-MCP and control plants (Fig. 4-1). However on day 7, a few buds had dropped prematurely from plants treated with 1-MCP. This may be due to incomplete binding of some receptor sites. Watkins et al. (2000) suggested this theory when they found partial responses in ripening delay in some apple cultivars. Another explanation may be that stage-1 buds are developing fast and the new receptors generated are not protected by 1-MCP (Blankenship and Dole, 2003).

By the end of observation period, no difference between the 1-MCP treatments and control was found in Stages-2, 3, 4 and 5 flower buds (Fig. 4-2, 4-3, 4-4 and 4-5). Ethylene treatment accelerated bud drop in all the developmental stages with the larger buds dropping first followed by younger buds. This result is consistent with results obtained in chapter 3 experiment 2 where by day 8 all bud stages abscised. 1-MCP prevented premature bud abscission on plants exposed to ethylene but had no effect in the absence of ethylene. This is in agreement with most cut and potted flower plants as 1-MCP had no effect in the absence of exogenous ethylene (Celikel et al., 2002; Elgar et al., 1999; Serek and Reid, 2000; Serek and Sisler, 2001).

Results of this experiment showed that ethylene-induced flower bud abscission was completely inhibited by 1-MCP when exposure to ethylene was followed by 1-MCP

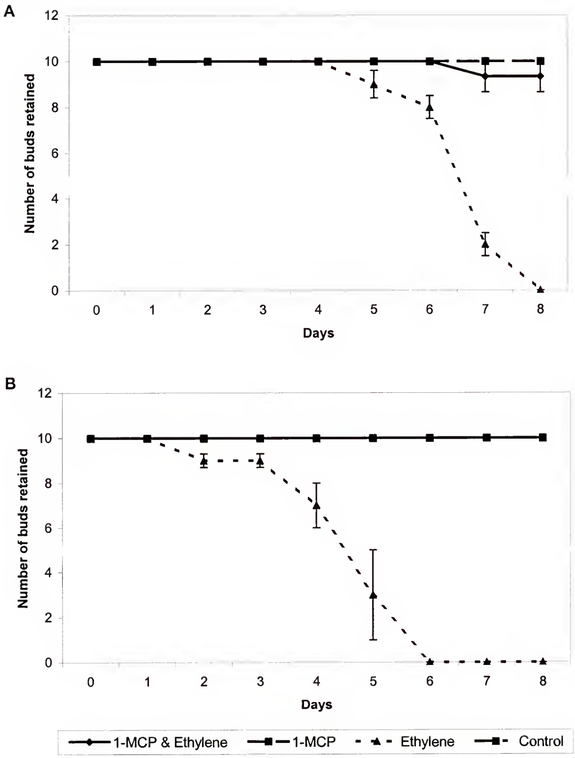

Figure 4-1 and 4-2. Total number of buds at 5 developmental stages; A. Stage 1 and B. Stage 2 retained on plants treated with 1-MCP and Ethylene, 1-MCP, Ethylene and Control

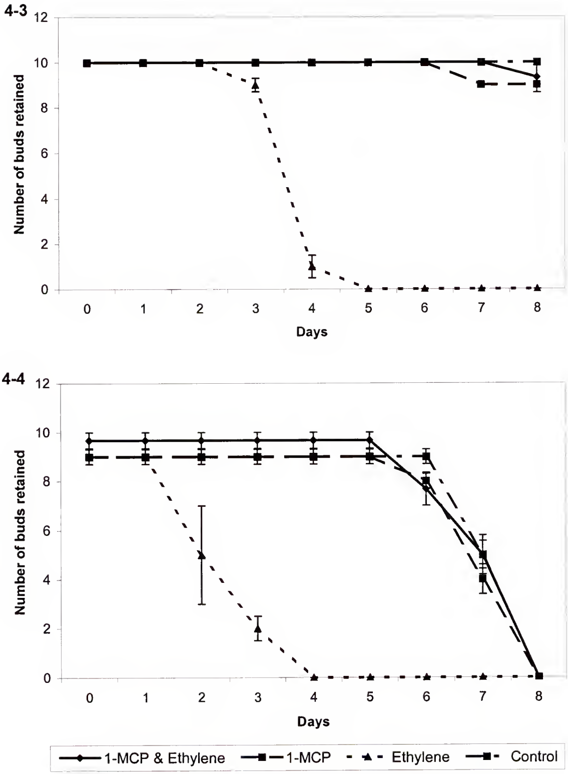

Figure 4-3 and 4-4. Total number of buds at 5 developmental stages; C. Stage 3 and D. Stage 4 retained on plants treated with 1-MCP and Ethylene, 1-MCP, Ethylene and Control

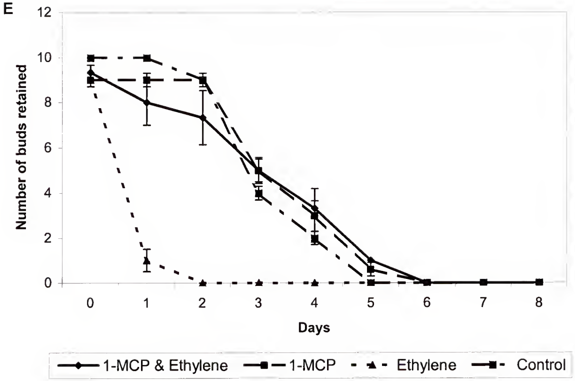

Figure 4-5. Total number of buds at 5 developmental stages; E. Stage 5 retained on plants treated with 1-MCP and Ethylene, 1-MCP, Ethylene and Control

treatment. However in plants not given exogenous ethylene there was no apparent beneficial effect of 1-MCP. Similar results have been found in other species (Celikel and Reid, 2002; Cameron and Reid, 2001).

Experiment 2: Duration of 1-MCP Effects

The objective of this experiment was to study the apparent decline in effectiveness in prevention of ethylene induced flower bud abscission as a function of time between 1-MCP and ethylene exposure. In all bud stages, 1-MCP dramatically reduced ethylene induced bud abscission when ethylene exposure was immediately after the 1-MCP treatment however at 2 or 4 days, excessive bud drop occurred (Fig. 4-6 to 4-10). Results showed a three way interaction between treatments, bud stage and observation day.

Significant difference in number of buds retained for all bud stages ($Pr < 0.0001$) was found between 1-MCP pretreated plants exposed to ethylene after 2 days and also after 4 days compared to pretreated plants exposed immediately to ethylene, 1-MCP only or control plants bud stages (Fig. 4-6 to 4-10). These results agree with the assumption that 1-MCP binds permanently to receptors present at the time of treatment and the damage caused by ethylene is due to appearance of new sites (Blankenship and Dole, 2003). Plants vary in their ability to regenerate sites and it seems that *H. rosa-sinensis* develop new sites after treatment which reduce its effectiveness to prevent ethylene damage. Results also showed that the effect of 1- MCP is not transient because the number of buds abscising increased with time after 1-MCP treatment. These results are in agreement with Cameron and Reid (2001) as they reported an increase in ethylene-induced petal abscission of *Pelargonium* with time after 1-MCP treatment. In the absence of ethylene, 1-MCP did not show any beneficial effect in extending the shelf life of the plant.

In general, 1-MCP binds to ethylene binding sites and disables those sites permanently (Sisler and Serek, 1997) as shown by the reduction in bud abscission in the presence of ethylene. Ethylene binding sites appear to be blocked shortly after 1-MCP treatment, however, the plant's sensitivity to ethylene increases with time after 1-MCP treatment. It is likely that with the plant development new sites, which are not blocked, were synthesized. In this experiment, the pattern of bud drop after ethylene exposure is consistent with our previous observation in experiment 3-1, with the larger buds abscising first followed by smaller buds.

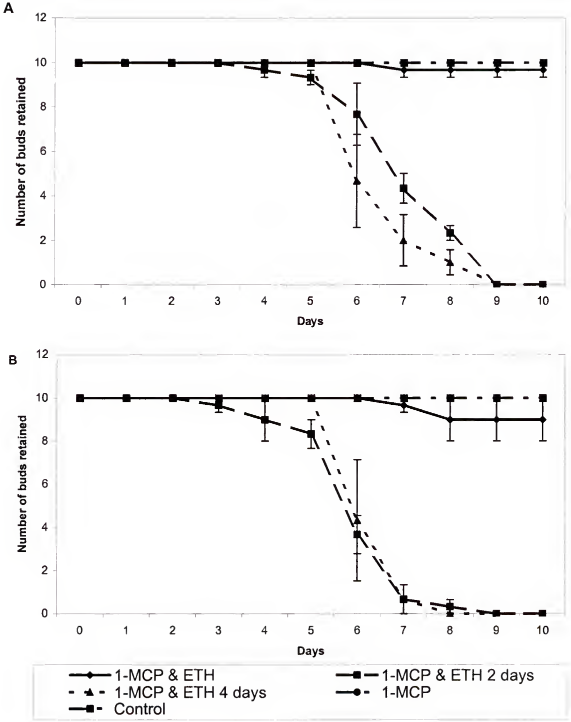

Figure 4-6 and 4-7. Total number of buds retained by 1-MCP pretreated plants as a function of time to ethylene ($1 \mu L.l^{-1}$) exposure (0, 2 and 4 days), 1-MCP alone and control for all developmental stages, A. Stage 1 buds and B. Stage 2 buds

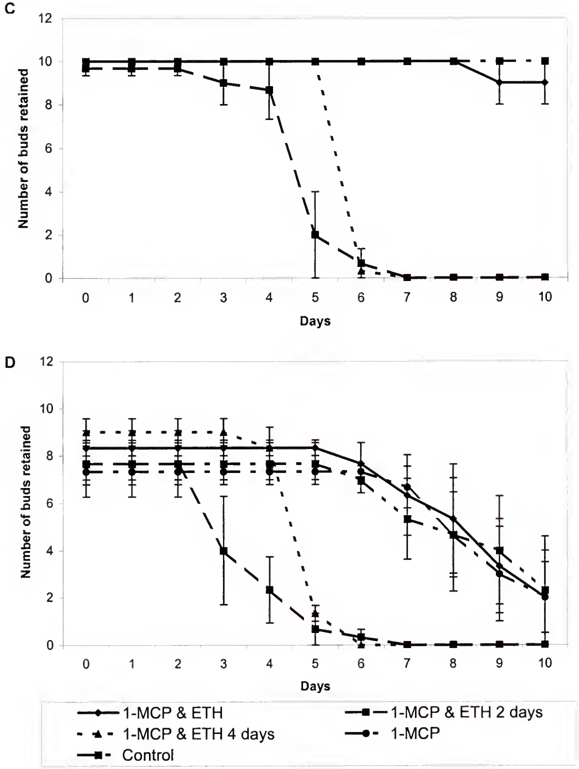

Figure 4-8 and 4-9. Total number of buds retained by 1-MCP pretreated plants as a function of time to ethylene ($1 \mu L.l^{-1}$) exposure (0, 2 and 4 days), 1-MCP alone and control for all developmental stages, C. Stage 3 buds and D. Stage 4 buds

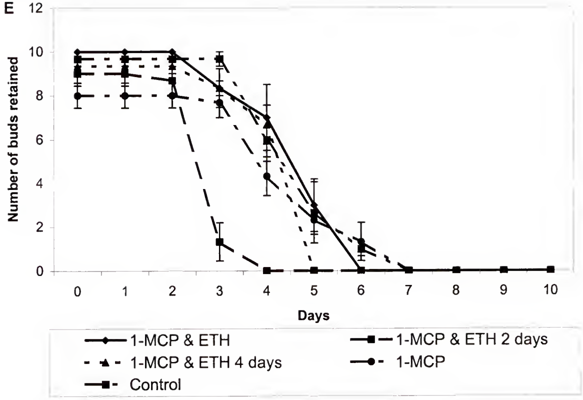

Figure 4-10. Total number of buds retained by 1-MCP pretreated plants as a function of time to ethylene ($1 \mu L.l^{-1}$) exposure (0, 2 and 4 days), 1-MCP alone and control for all developmental stages, E. Stage 5 buds

Experiment 3: The Use of Ethylene Blockers in Shipping

The 3-way interaction of shipping duration, ethylene blockers treatments and bud stage was significant (< 0.0001). In plants shipped for 4 days, a significant difference between the ethylene blockers treatments could be seen for Stage 1, 2 and 3 buds (Fig. 4-11, 12 and 13, respectively). Whereas on Stage 4 (Fig. 4-14) and 5 buds none of the treatments had a significant effect as they flowered and dropped during shipping. Results show that bud drop of Stage 1 and 2 from plants treated with 1-MCP was initially slower than on control plants as shown in figure 4-11 and 4-12. However less than 10% of Stage 1 buds were retained on 1-MCP treated plants on day 8, only one day before control plants reached a similar level of bud retention. 1-MCP plants had 30 % Stage 2 buds retained on day 10 compared to control plants which had less than 10 % buds retained on the same day. STS likewise delayed initial loss of Stage 1 buds but those plants reached less than 10% retention on day 7, as did the control. Similarly, Stage 2 buds on STS plants as well as control plants showed an initial delay in bud abscission but on day 7, bud retention reached less than 10%. STS caused abscission of Stage 3 buds where 50% of the buds were retained on day 6 compared to the other treatments (1-MCP, AVG and control plants), which had 100% of the buds (Fig. 4-13).

AVG also delayed initial abscission of Stage 1 buds on treated plants, but there was considerable loss of buds between day 6 and day 9 (Fig. 4-11). Unlike plants treated with STS and 1-MCP, at day 12 the AVG plants retention approximately 40% of the Stage 1 buds, and these buds continued to develop normally through flower opening. A similar pattern was observed on Stage 2 buds on AVG treated plants where initially bud abscission was delays but bud drop increased between day 6 and 9 (Fig. 4-12).

Shipping duration of 4 days or longer has been previously shown to cause abscission (Nell and Barrett, 1984; Cushman et al., 1998). For 6 days shipping Stage 1 and 2 indicate that STS and 1-MCP had only a slight effect on bud drop and more than 90% abscised by day 8 (2 days after removal from the shipping treatment) and similar to the pattern observed on 4 days shipping (Fig. 4-15 and 4-16). Stage 1 and 2 buds responded favorably to AVG treatment on plants shipped for 6 days where initial delay was shown. However 80% of Stage 1 buds and 70 % of Stage 2 buds abscised on day 12, unlike the remaining treatments (STS, 1-MCP and control plants) that had less that 10% of their buds. It appears that under these shipping conditions ethylene synthesis is not completely blocked by AVG, which ultimately cause some of the buds to abscise. No difference between the shipping durations was observed on the larger buds (Stage 3, 4 and 5 buds) since they continued to develop normally during the shipping period and went through natural senescence and subsequent abscission (Fig 4-17). The effect of shipping on larger buds is similar to the results observed earlier in a previous experiment as well as results reported by Force et al. (1988). According to Environmental Protection Agency (EPT) fact sheet (1997), AVG reduced shipping related abscission on certain ornamentals such as miniature carnation and *H. rosa-sinensis*.

Silver thiosulphate did not give the anticipated protection during shipping for all bud stages. Result of this study did not agree with previous studies on STS and shipping of *H. rosa-sinensis* (Høyer, 1986; Thaxton et al., 1988) as they reported a reduction in abscission during shipping when plants were pretreated with STS. In the study by Høyer (1986), *H. rosa-sinensis* plants were sprayed with 0.45 or 4.05 mM STS 11 days before simulated shipping in darkness for 72 hrs at 21°C. Following the shipping treatment plants were placed in an interior environment at 20°C for 12 days observation. Høyer (1986) reported that treatment of *H. rosa-sinensis* with STS prevented bud drop to a certain extent but the remaining buds became yellow and did not develop. Phytotoxicity in the form of injured flowers was observed on plants treated with 4.05 mM STS following simulated shipping but not before. The injurious effect of STS was found mainly on plants exposed to darkness and high humidity but did not appear in plants kept in the greenhouse.

Thaxton et al. (1988) also studied the effect of STS on flower bud abscission of *H. rosa-sinensis* cv. 'Brilliant Red' by spraying the plants with 0 or 4 mM STS 7 days prior to simulated shipping. Plants from both spray treatments were placed in paper sleeves

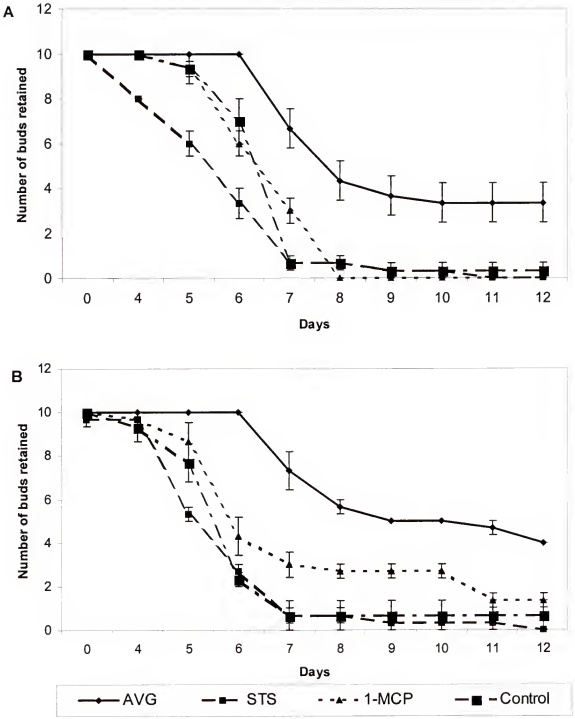

Figure 4-11 and 4-12. Total number of buds retained by plants pretreated with AVG, STS, 1-MCP and Control then shipped for 4 days. A. Stage 1, B. Stage 2

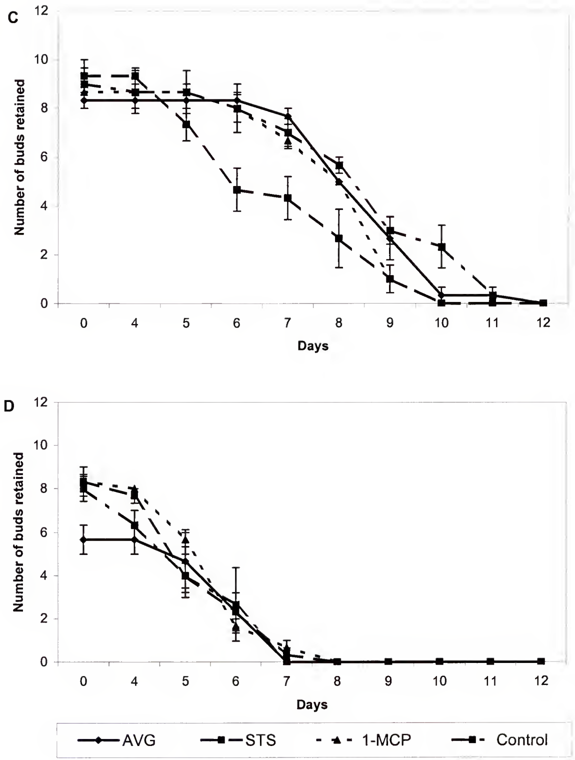

Figure 4-13 and 4-14. Total number of buds retained by plants pretreated with AVG, STS, 1-MCP and Control then shipped for 4. C. Stage 3 and D. Stage 4

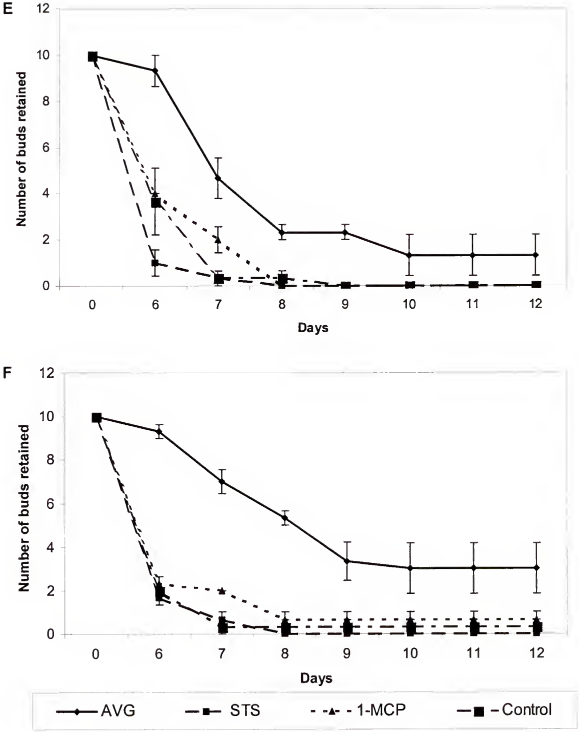

Figure 4-15 and 4-16. Total number of buds retained by plants pretreated with AVG, STS, 1-MCP and Control then shipped 6 days. E. Stage 1 and F. Stage 2

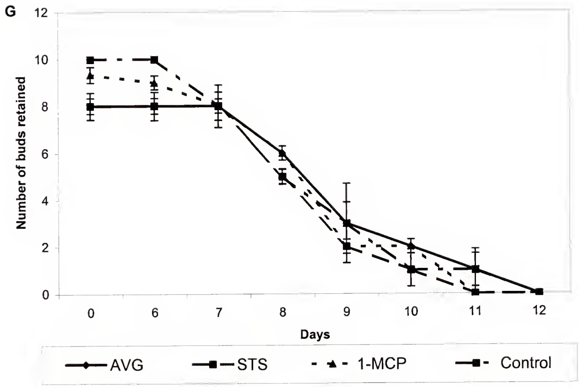

Figure 4-17. Total number of buds retained by plants pretreated with AVG, STS, 1-MCP and Control then shipped 6 days. G. Stage 3

and then placed in chambers at 10, 20 or 30° C. After removal from shipping plants were placed in a simulated office environment where abscission rate was monitored. Thaxton reported that STS was effective in reducing bud abscission at all temperatures when sprayed 7 days prior shipping. The shipping environment used in the present study, the cultivar used and/or the concentration of STS used may have attributed to the bud loss.

Results obtained from this experiment show that AVG protected the smaller buds to a certain extent and reduced the effect of shipping, while STS and 1-MCP did not protect the shipped plants. Experiments on individual ethylene blocks appeared to be necessary to clarify the results reported in this experiment.

Experiment 4: 1-MCP and Shipping

Comparing the treatments on day 6 showed a significant effect (Pr <0.0057) of treatment and bud stages (Table 4-2). The difference was mainly between the developmental stages where stage 1 to Stage 4 buds retained more buds by day six compared to Stage 5. Treating the plants with 1-MCP prior to shipping did not prevent bud abscission of Stage 1 and 2 and no difference was found between 1-MCP and control plants. A significant effect was also found between treatment and shipping duration (0.0068). Plants shipped for 4 days caused less abscission than those shipped for 6 days. Stage 3 and larger buds abscised after going through natural senescence. These results are in agreement with experiment 3 indicating that during shipping continuous development of the buds regenerate new receptor sites, which are not protected against endogenous ethylene.

Experiment 5: STS and Shipping

In a preliminary experiments (date not shown) and experiment 3 (Fig. 4-11 to 4-17) where the plants were shipped at constant temperature of 25° C, STS had no significant effect in preventing bud abscission of all stages. These results contradicted previous studied done on *H. rosa-sinensis* where STS reduced bud abscission during dark shipping treatments (Høyer, 1986; Force et al., 1988). The assumption was made that either the shipping environment (i.e. temperature), the cultivar or the concentration may be involved in causing the buds to abscise therefore limiting the effect of STS. This experiment was conducted to try and determine the effect of STS on flower bud abscission during shipping at a lower temperature (20° C). On plants shipped for 4 days a 3-way interaction was found between chemical treatments, bud stages and observation time ($Pr < 0.0001$). STS reduced the number of buds abscising compared to control

Table 4-2. Total number of buds retained on day 6 for the two shipping durations (4 and 6 days) and two treatments Control and 1-MCP

Treatment	Shipping	Stages					Main Effects(1)
		1	2	3	4	5	
Control	4	9	10	9.6	6.0	0	6.9
	6	5	7.3	8.6	2.3	0	4.6
	Main Effects(2)	7	8.6	9.1	4.2	0	
1-MCP	4	9.6	9.0	8.6	5.3	0	6.5
	6	8.3	7.6	7.3	6.3	0	5.9
	Main Effects(2)	9	8.3	8	5.8	0	
Pr > F							
Treat	0.1452 ^{ns}						
Stage	<0.0001 ***						
Treat*Stage	0.0057 *						
Ship	<0.0001***						
Treat*Ship	0.0068 *						
Stage*Ship	0.0723^{ns}						
Treat*Stage*Ship	0.0596^{ns}						

(1) Means pooled over stages; HSD=1.1
(2) Means pooled over shipping; HSD=2.18
$HSD_{0.05}$ = Honest Significant Difference at 0.05 Probability
ns: not significant, * significant at 0.05, *** significant at 0.0001

plants (Fig. 4-18). STS completely prevented the abscission of Stage 1 buds. Stage 1 buds on control plants began to abscise on day 6 and by day 12 the control plants retained 80% of its buds while STS treated plants had 100%. STS reduced the abscission of Stage 2 buds where initially there was a delay in bud drop but by day 11 some buds dropped. Approximately 78% of Stage 2 buds were retained by day 12 compared to control plants where only 40% was retained. Stage 3 buds did not show a big difference between STS and control plants since they continued to develop normally and go through senescence and abscission.

Similarly a significant difference (Pr < 0.0001) was found on plants shipped for 6 days (Fig 4-19). STS was able to completely prevent bud drop on Stage 1 buds while on control plants there were approximately 30% of bud retained. The treatment also reduced abscission of Stage 2 buds where plants treated with STS retained approximately 95% of their buds by day 12 whereas on control plants 70% was retained. Stage 1 and 2 appear to benefit from STS treatment whereas Stage 3 buds and larger continues to develop normally. Results of this experiment are in agreement with previous studies on STS. Temperature during shipping appears to determine the effectiveness of STS to bud abscission.

Experiment 6: AVG and Shipping

An over all interaction (< 0.0001) between treatment (AVG and control), temperature (20 and 25° C), shipping duration (3 and 6 days), bud stages and observation time was found. AVG treated plants shipped for 3 days had no bud abscission of Stage 1 bud at both temperatures compared to control plants where some loss of buds was observed on plants shipped at lower temperature (20° C), while bud loss was extensive on control plants shipped at the higher temperature (25° C) (Fig. 4-20). AVG treatment also prevented bud abscission of Stage 2 buds shipped at lower temperature but reduced the bud loss on the plants shipped at higher temperature where only 50 % of the buds were retained by day 12. Similar to results observed in the previous experiment, there was an initial delay in bud loss on Stage 2 buds where bud loss began on day 9 (Fig. 4-21). Larger buds (Stage 3, 4 and 5 buds) did not show much

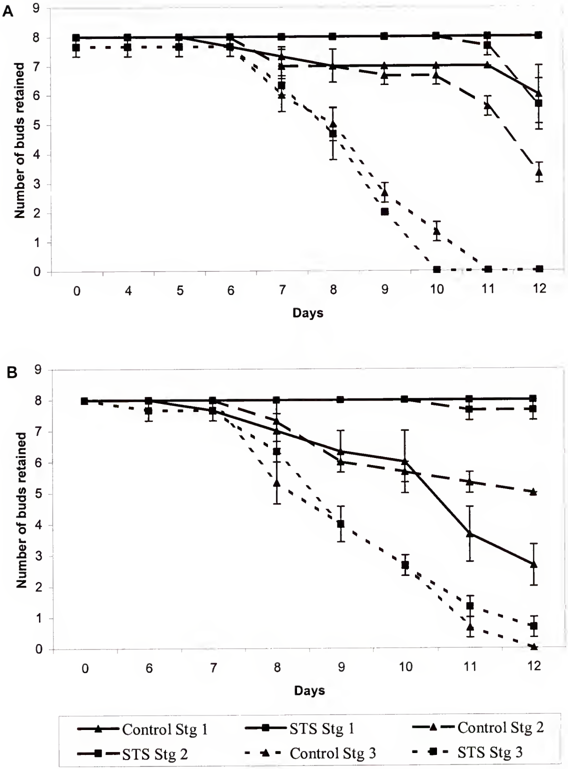

Figure 4-18 and 4-19. Total number of buds retained on plants pretreated with STS and control for three bud stages (1, 2 and 3) then shipped for A. 4 days and B. 6 days benefit of AVG at both temperatures as the buds continued to develop normally during shipping until flowering and senescence (Fig. 4-22 and 4-23). Shipping the AVG treated plants for 6 days prevented bud abscission of Stage 1 at lower temperature but not at the higher temperature (Fig 4-24). At higher temperature, plants treated with AVG initially had delayed bud drop of Stage 1 buds however less than 10% were retained on day 12. However control plants had excessive loss of Stage 1 buds especially when shipped at higher temperature with plants losing all their Stage 1 buds on day 8. Likewise there was some loss of Stage 2 buds at lower temperature shipping on AVG treated plants but considerable loss was observed on plants shipped at higher temperature with 80% of buds retained by day 12 (Fig. 4-25). Similarly, excessive bud loss was recorded on control plants shipped at both temperatures. Stage 3 and larger buds did not benefit from the AVG treatment as the buds continued to develop normally as have been reported previously with buds shipped at higher temperature developing (Fig. 4-26 and 4-27).

Shipping temperature appears to have an important role in bud abscission. It has been reported that shipping at high temperature decrease the plants quality by increasing bud and leaf abscission (Nell and Barrett, 1984; Conover and Poole, 1984). High temperatures also increase the development rate of the plant, which in turn does not allow the buds to respond to the ethylene blokers treatments. In a cooler shipping environment AVG completely prevent bud abscission even at longer duration. This may be due to the reduction in the amount of ethylene synthesized by the plant in cooler temperatures. Supporting this claim is the reduction in bud abscission on control plants. But even though the AVG treated plants are protected from endogenous ethylene, any exposure to exogenous ethylene will cause buds to abscise. In agreement, Staby et al. (1993)

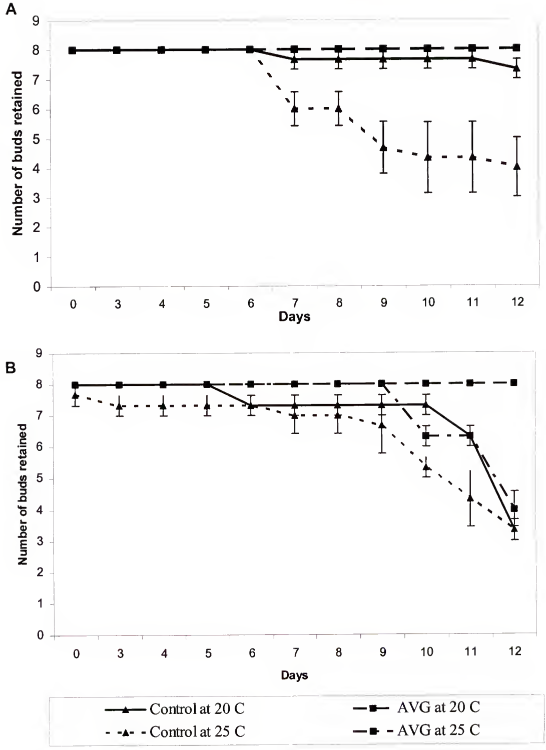

Figure 4-20 and 4-21. Total number of buds retained of the five developmental stages: A. Stage 1 and B. Stage 2 buds on control and AVG treated plants shipped for 3 days at 20 or 25° C

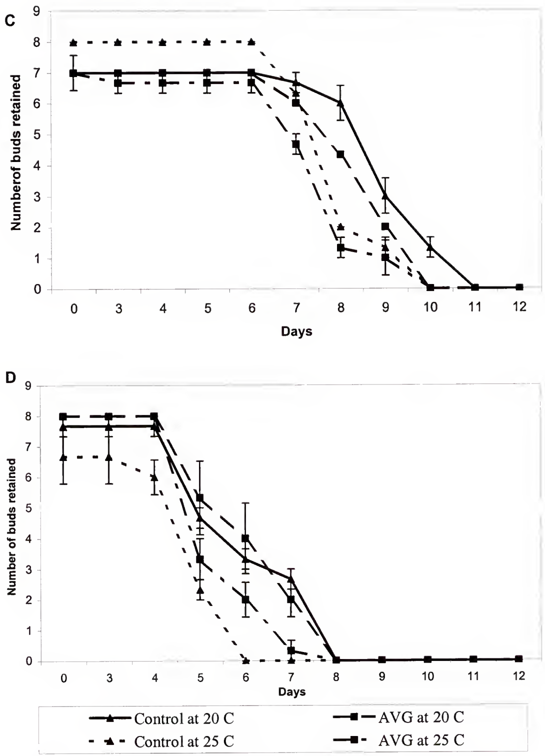

Figure 4-22 and 4-23. Total number of buds retained of the five developmental stages: C. Stage 3 and D. Stage 4 buds on control and AVG treated plants shipped for 3 days at 20 or 25° C

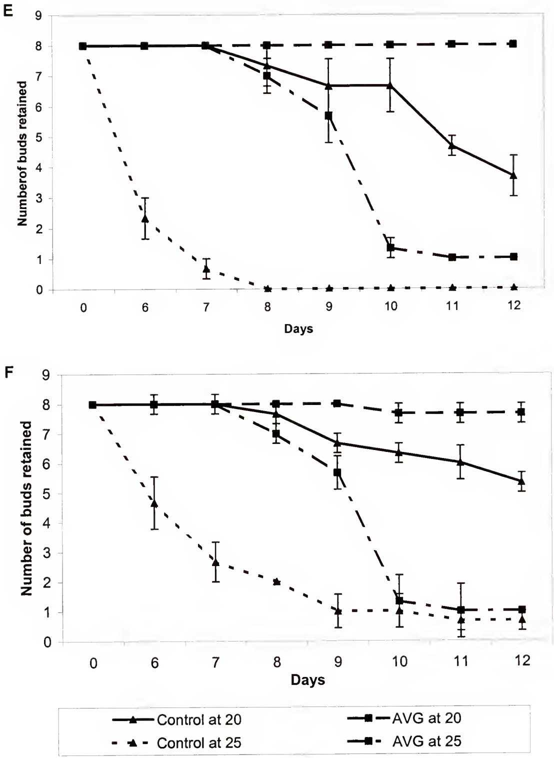

Figure 4-24 and 4-25. Total number of buds retained of the five developmental stages: E. Stage 1 and F. Stage 2 buds on control and AVG treated plants shipped for 6 days at 20 or 25° C

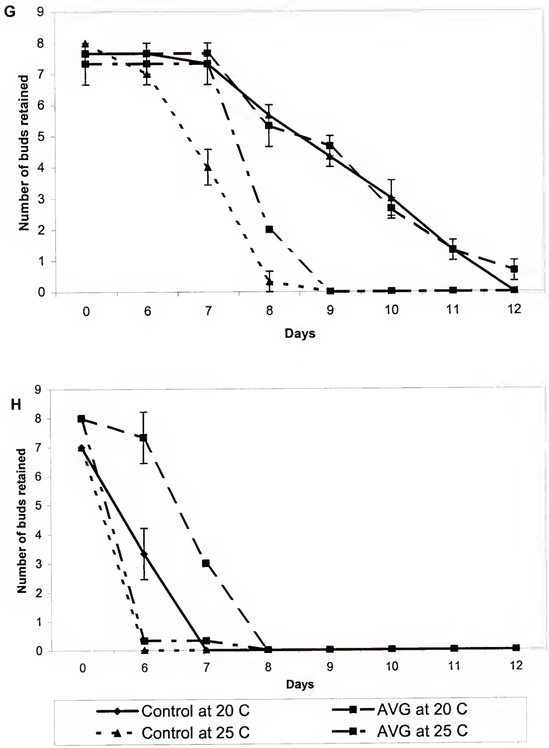

Figure 4-26 and 4-27. Total number of buds retained of the five developmental stages: G. Stage 3 and H. Stage 4 buds on control and AVG treated plants shipped for 6 days at 20 or 25° C reported that exposing AVG treated plants to several ethylene levels offered little or no protection against ethylene damage.

Experiments from this study show clearly that ethylene is at least partially involved in dark shipping induced bud abscission. Ethylene blockers provide protection from excessive bud abscission of Stage 1 and 2 buds but only if shipped at low temperatures. AVG provided the most protection for the smaller buds during shipping compared to 1-MCP and STS treatments however since AVG does not protect against exogenous ethylene its commercial significance is doubted.

CHAPTER 5

CHARACTERIZATION OF CAYLX AND PEDUNCLE ABSCISSION ZONES OF *H. rosa-sinensis* FLOWERS

The abscission zone develops within a small area of specialized cells, which are anatomically similar to adjacent cells (Sexton and Roberts, 1982). Cells of the abscission zone are the only ones capable of responding to inductive stimuli leading to separation (Zanchin et al., 1995). During normal abscission, cell wall dissolution and other changes occur in the abscission zone and a separation layer develops (Webster, 1968; Sexton and Roberts, 1982). In some plant species, such as *Phaseolus* and *Coleus*, cell division precedes cell wall dissolution (Sexton et al., 1977), while in others no cell division is found, e.g. flowers of *Impatiens sultani* and *Gossypium hirsutunm* (Reed and Hartmann, 1976; Dutt, 1928). Ethylene treatments did not induce cell division in the abscission zone of *Euphorbia pulcherrima* and *Gossypium herbaceam* (Gawadi and Avery, 1950) but did induce the synthesis of cell wall hydrolases causing premature cell separation in *Prunus persica* (Zanchin et al., 1995).

Multiple factors, such as temperature, water stress and ethylene, induce abscission (Dewit and Reid, 1992; Zanchin et al., 1995). Response to these influences varies between different organs of the same plant as well as different abscission zones. In a number of plant species, bean leaves (del Campillo et al., 1990), olive inflorescence (Weis et al., 1988) and peach flower/fruit (Zanchin et al., 1995), multiple precisely positioned abscission zones have been observed.

The *H. rosa-sinensis* flower has three abscission zones: (1) petal abscission zone, located at the base of the petals; (2) calyx abscission zone, located approximately 1-1.5 cm beneath the calyx; (3) peduncle abscission zone is found at the point of connection between the peduncle and the main stem (Fig. 5-1). The calyx abscission zone is marked by a faint external groove or indentation. This groove is visible in mature flower bud pedicles but is very faint in younger buds. A similar abscission zone is found in the cotton flower, but is usually located at or near the base of the peduncle (Dutt, 1928) and in the tomato pedicle (Tabuchi et al., 2001). In *H. rosa-sinensis*, only the calyx abscission zone has been studied during normal abscission (Gilliland et al., 1976). In a previous study (chapter 3) the peduncle abscission zone appears to be activated only after the calyx has dropped and detailed information on the activation of this abscission zone is not available. The effect of ethylene on the peduncle abscission zone has not been documented. This investigation was designed to determine the anatomical changes occurring in the calyx and peduncle abscission zones of *H. rosa-sinensis*, the relationship between the calyx and the peduncle abscission zone and also the effect of ethylene on the peduncle abscission zone.

Materials and Methods

A series of experiments were conducted to examine the calyx abscission zone and peduncle abscission zone of *H. rosa-sinensis* 'Pink Versicolor'. Rooted cuttings in Oasis media, were obtained from a commercial nursery (Yoder Brothers, Parrish, FL) and immediately transplanted into 10-cm (600-ml) plastic pots containing a commercial media (Fafard mix # 2, Conrad Fafard Inc, MA). After transplanting the plants were transferred to a temperature controlled double polyethylene greenhouse maintained

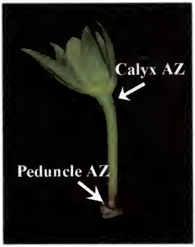

Figure 5-1. Stage 4 bud showing the positions of the calyx abscission zone (AZ) and the peduncle abscission zone

between 26-30° C day and 21-24°C night temperatures under natural photoperiods. Plants were fertigated daily via drip irrigation with using a 20 N – 4.4 P – 16.6 K with N concentration of 150 mg.L^{-1} (Scott's Peters fertilizer, Scott's –Sierra Horticultural Products Co., OH). Two weeks after potting, the plants were pinched to approximately three nodes and then treated with 21 ml.L^{-1} [chlormequat chloride (2-chloroethyl) trimethylammonium chloride] (Cycocel), to obtain compact growth. The plants were allowed to flower freely under greenhouse conditions with no further treatments. All open flowers were removed daily from each plant until a week before the beginning of the experiment and then subsequent flowers were allowed to develop and senesce naturally. In this study the flower bud development was divided into six different developmental stages ranging from very young buds (Stage 1) to the opening flower (Stage 6) (Table 5-1). In addition, the stage following petal senescence (S1) and abscission (Abs 1) was observed.

Table 5-1. Developmental stages of floral buds on *H. rosa-sinensis*

Bud Stages	Description
Stage 1	Very young bud with calyx not parted -< 1 cm in length
Stage 2	Calyx is slightly open and bud is between 1 and 2 cm in length
Stage 3	Expanding bud with calyx completely open but no corolla visible
Stage 4	Pigmented corolla is just visible, but less than ¼ of corolla is showing
Stage 5	More than ¼ of the corolla is visible, but the corolla is still rolled tight
Stage 6	Open flower
S1	One day after flower petal senescence
Abs 1	One day after buds abscise following senescence

Experiment 1: Calyx Abscission Zone During Pre-Abscission Period.

Greenhouse-grown plants were used as an explant source. Flower buds of the different developmental stages were removed from the plants and placed in distilled water. Samples containing the calyx abscission zones were prepared by removing a 10 mm section of the pedicel containing the calyx abscission zone (7 mm proximal to the abscission zone and 3 mm distal to it). Using a double bladed razor, a longitudinal section was made in the middle of the sample. The sections were then placed on a slide and covered with glycerin and water before adding a slide cover. The slides were viewed using a Polarized Nikon light microscope equipped with a camera. Observations were done on all bud stages in addition to S1 (one day following petal senescence). The experiment was done in January and February 2003. There were at least five buds per stage and the experiment was repeated three times.

Experiment 2: Relationship Between Calyx and Peduncle Abscission Zone.

Stage 2 and 4 flower buds on greenhouse grown plants were removed approximately 5 mm either above or below the calyx abscission zone (Fig. 5-2). Samples were then collected 2, 3 and 4 days later. Each sample included a portion of the stem above and below the calyx abscission zone. Samples were placed in distilled water to prevent dehydration before transporting to the laboratory. In the laboratory the stem sections were cut 5 mm above and below the peduncle abscission zone (Fig. 5-3). The fresh sections were prepared and observed as described in experiment 1. This experiment was conducted in September, 2003 and repeated 3 times.

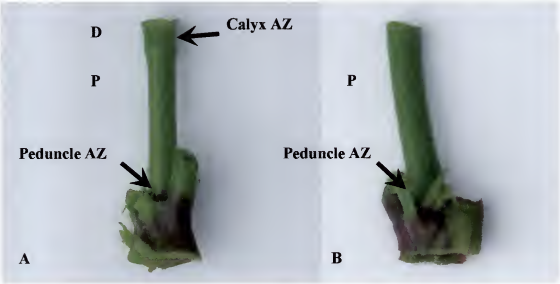

Figure 5-2. The pedicel after the removal of the calyx and epicalyx A. above the calyx abscission zone and B. below the calyx abscission zone. P: proximal, D: Distal

Experiment 3: Ethylene Effects on The Peduncle Abscission Zone

The time interval for differentiation of the peduncle abscission zone in response to ethylene exposure was determined as well as the effect of ethylene on the peduncle abscission zone. The following treatments were conducted during September and October 2003:

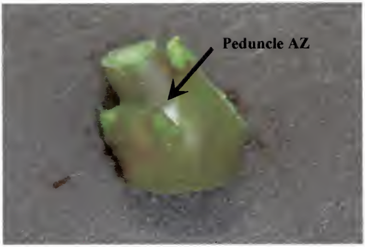

Figure 5-3. The prepared sample of peduncle abscission zone before sectioning showing a portion of the stem above and below the peduncle abscission zone

1. Plants were treated with ethylene at 1 $\mu L.l^{-1}$ for 24 hours in the dark at 20° C. Plants were placed in an airtight glass aquarium and ethylene was injected into the chamber in an airflow method exposing the plants and flower buds to ethylene. Ethylene levels were checked by extracting gas samples from the chambers and using gas chromatography (Hewlett Packard 5890 Series II). After treatment, the potted plants were removed from the chambers and placed in a greenhouse and flower buds were collected.

2. Individual calyx and peduncle abscission zones of stage 2 and 4 buds were treated with 500 mg. L^{-1} of Ethephon (2-Chloroethyl) phosphonic acid (Southern Agricultural Insecticides Inc., Hendersonville, NC) while still attached to the plants grown in the greenhouse. A cotton swab was used to apply the ethephon solution directly on the abscission zone. Each flower bud was either treated at the calyx or peduncle abscission zone but not both. The abscission zones were observed during a period of five days where any changes were recorded. A sample was also taken of both abscission zones every day following the treatment. Sections of the calyx abscission zone could not be made due to difficulties in handling the sample. Separation of the calyx abscission zone occurred before the sections could be taken.

In both treatments the peduncle abscission zone was sectioned and observed under the light microscope as previously described.

Results and Discussion

In chapters 3 and 4, the effects of shipping and ethylene on flower bud abscission of *H. rosa-sinensis* were reported. Abscission in *H. rosa-sinensis* was previously documented in response to shipping (Nell and Barrett, 1984; Gibbs et al., 1989; Van Meeteren and Van Gelder, 1995) and to ethylene (Nell and Barrett, 1984; Force et al., 1988; Thaxton et al., 1988; Van Meeteren and Van Gelder, 1995, 2000; Høyer, 1996). However, these reports did not describe which of the three abscission zones were affected. As shown in Chapter 3, stages 1 and 2 flower buds on *H. rosa-sinensis plants* placed in shipping or treated with ethylene abscised at the peduncle abscission zone; whereas, the larger buds (stage 3 buds and larger) abscised at the calyx abscission zone when slight pressure was applied on the bud. Preliminary studies revealed that on plants treated with ethylene at 1 $\mu L.l^{-1}$ for 24 hours and placed undisturbed in the greenhouse buds at all stages abscised at the peduncle abscission zone after yellowing of the bud. Based on these observations, the following experiments were planned to gain an insight on the process of abscission as well as the relationship between the calyx abscission zone and peduncle abscission zone.

Experiment 1: Calyx abscission zone during pre-abscission period.

Figure 5-4 shows the calyx abscission zone for Stage 1 and Stage 5 buds. The calyx abscission zone appeared to be a morphological feature at an early developmental stage (Stage 1) with the dark colored groove becoming more distinctive as the bud matures. Cell division in the abscission zone continued up to the time of abscission. The vascular tissues in the abscission zone especially at the separation layer were less developed compared to development in adjacent tissues. This weakening of the vascular tissue allows the separation to occur without application of mechanical force. Observations of the calyx abscission zone with a light microscope in this study were similar to those reported by Gilliland et al. (1976).

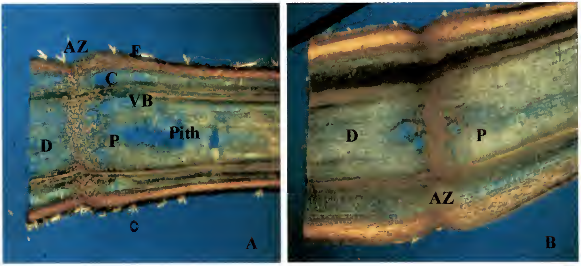

Figure 5-4: Calyx abscission zone of Stage 1 (A) and Stage 5 (B) buds. The abscission zone is well developed by Stage 1. Continuous cell division occurs during the development as seen in the Stage 5 abscission zone. Calcium oxalate is found in the abscission zone. Distal (D) and Proximal (P) orientation is shown and the vascular bundle (VB), cortex (C) and epidermis (E) are indicated.

The separation layer formed 3 or 4 layers distal to the groove and as separation approached the cells distal to the abscission zone became slightly elongated. Separation of the tissue occurred soon after the separation zone was formed. The separation started at the epidermis and progressed inward towards the cortex, through the vascular tissue and then the pith (Fig. 5-5). Huberman et al. (1983) described this as one of several ways by which separation occurs in abscission zone. This process has been found in citrus pedicel (Jaffe and Goren, 1979), in the bud-peduncle of *Begonia* (Hanishten Cate et al., 1975) and the pedicel of soybean (Oberholster et al., 1991). Separation of *H. rosa-sinensis* involved only the middle lamellae and cells remained intact. Similarly separation of the of the bean leaflet (Sexton, 1995) and cotton leaf and flower (Dutt, 1928) occurred along the middle lamella, leaving intact rounded cells covering the surface.

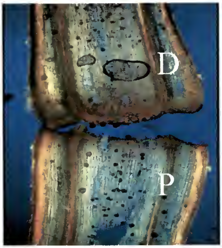

Figure 5-5: Separation of the calyx abscission zone following flower senescence showing the separation occurring 3 or 4 layers distal to the groove and the separation staring at the epidermis and progressing inward. Proximal (P)and Distal (D) orientation is shown.

Numerous calcium oxalate crystals were observed in the abscission zone and as the bud developed the number of crystals increased (Fig. 5-4). Gilliland et al. (1976) also reported calcium oxalate crystals in *H. rosa-sinensis* and hypothesized that the calcium released by the hydrolysis of the cell wall pectins caused the increase in calcium oxalate crystals. The exact function of the crystals in *H. rosa-sinensis* abscission zone has not been reported, however it is speculated that calcium oxalate provides a relatively insoluble, metabolically inactive salt for excessive calcium (Kinzel, 1989). Therefore, calcium oxalate is considered to be a high capacity storage compound in the plant (Webb, 1999). Calcium oxalate was also found in abscission zones of *Pennisetum glaucum* (Heslop-Harrison and Heslop-Harrison, 1997) and lemon leaf (Iwahori and Van Steveninck, 1989).

This investigation showed that the calyx abscission zone of *H. rosa-sinensis* is preformed early in the bud development, but it does not become functional until induced by senescing of the petals.

Experiment 2: Relationship Between Calyx and Peduncle Abscission Zone

Normally the peduncle abscission zone does not become functional until the calyx abscission zone was induced either by flower senescence or ethylene (chapter 3). When the bud was removed above the calyx abscission zone no changes were detected in the peduncle abscission zone for four days (Fig. 5-6). However, when the bud was removed below the calyx abscission zone, abscission of the pedicel was induced at the peduncle abscission zone. Abscission of the remaining pedicel occurred approximately 5 days later. This was observed on both Stage 2 and Stage 4 buds. However when the bud was removed above the calyx abscission zone the peduncle remained intact through day 5. The peduncle abscission zone was eventually induced after the proximal portion above the calyx abscission zone dried and abscised at the calyx abscission zone. Observations under the light microscope showed that the peduncle abscission zone was not preformed (Fig. 5-7). When the bud was removed below the calyx abscission zone the peduncle abscission zone separation layer was microscopically determined on day 3 (Fig. 5-8) and abscission zone formation continued until the actual separation occurred on day 5. The separation layer is about one or two cell layers thick and it appeared to involve the middle lamellae (Fig. 5-9). Lloyd (1920) found that in young cotton bolls only one layer of cells was involved in abscission.

The separation layer was found at the junction between the peduncle and the stem where a macroscopically detectable groove is seen (Fig. 5-3). Similarly in young cotton

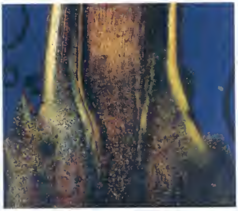

Figure 5-6: Longitudinal section of the peduncle abscission zone from a Stage 2 bud 3 days following the removal of the bud above the calyx abscission zone. No preformed abscission zone is present.

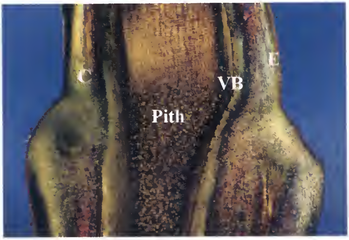

Figure 5-7: Longitudinal section of the peduncle abscission zone showing that the zone is not preformed. The vascular bundle (VB), cortex (C), epidermis (E) and pith are indicated.

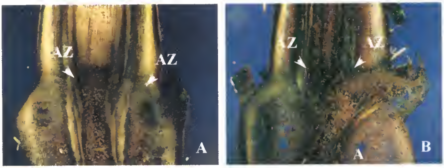

Figure 5-8: Longitudinal section of the peduncle abscission zone from a Stage 2 (A) and Stage 4 (B) 3 days following the removal of the bud below the calyx abscission zone. Arrows indicate the abscission zone (AZ) separation layer

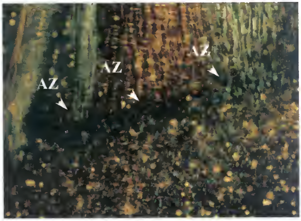

Figure 5-9: Close up of longitudinal section of the peduncle abscission zone from a Stage 4 bud 3 days following the removal of the bud below the calyx abscission zone showing the separation of the abscission zone, Arrows indicate the abscission zone (AZ) separation layer

leaves a slight depression is macroscopically visible in the area where the abscission zone will form (Leinweber and Hall, 1959). No evidence of cell division in the peduncle abscission zone of *H. rosa-sinensis* was detected prior to and during abscission, and this was also reported in cotton flower (Dutt, 1928). As in the calyx abscission zone, the actual separation of peduncle abscission zone begins at the epidermis and progresses inward towards the pith, however the abscission zone became microscopically detectable from the pith progressing outwards. Balls (1912), reported that the separation layer of cotton began in the cortex and extended inward with dividing walls between the cells splitting along the middle lamella. These observations were noted on the *H. rosa-sinensis* peduncle abscission zone of Stage 2 and 4 buds removed below the calyx abscission zone. The abscission zone can be activated in response to a specific trigger at a certain stage of development (Greenberg et al., 1975) as observed with the peduncle abscission zone.

Activation of the peduncle abscission zone was only induced when the calyx abscission zone was no longer attached to the pedicel. Therefore a close relationship between the two abscission zones exists, as the calyx abscission zone triggers the induction of peduncle abscission zone. This is clearly seen during the natural senescence of the flower where as long as the calyx remains attached the peduncle abscission zone does not develop then soon after the calyx abscission zone becomes functional and separates the peduncle abscission zone is induced. Calcium oxalate crystals were also observed in the peduncle abscission zone but the quantity was much less than that found in calyx abscission zone. There did not appear to be an increase of the crystals with the bud development.

Experiment 3: Ethylene Effects on Peduncle Abscission Zone

Ethylene accelerates abscission of *H. rosa-sinensis* flowers and flower buds (chapter 3, Høyer, 1996); however, the exact effect of ethylene on the individual abscission zones has not been reported. In a preliminary study, attempts were made to examine the calyx abscission zone following ethylene treatment but the handling of the abscission zone was difficult due to the occurrence of separation while sectioning. From visual observations the calyx abscission zone showed reaction to ethylene exposure by swelling and then becoming discolored one day following the treatment. Therefore this experiment was designed to determine the effect of ethylene on the peduncle abscission zone.

When plants were treated with 1μL.l^{-1} ethylene at 20° C for 24 hours the peduncle abscission zone did not show any changes on the day following removal from ethylene treatment. This was also observed in the abscission zone of peach (*Prunus persica*), where the peduncle and flower receptacle abscission zone could not be distinguished morphologically prior to ethylene treatment (Zanchin et al., 1995). On day 2, the future separation layer became microscopically detectable beginning initially at the pith and progressing outward across the vascular strands to the cortex prior to abscission of the peduncle (Fig. 5-10). The actual separation took place 4 days after the ethylene treatment. This was detected on both Stage 2 and 4 buds.

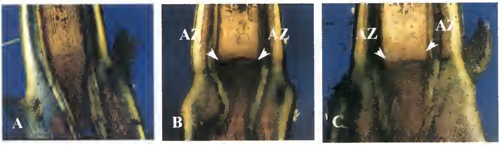

Figure 5-10: Longitudinal section of the peduncle abscission zone following ethylene treatment. A is a Stage 2 bud one day after the treatment showing no separation layer. B is a Stage 2 bud and C is a Stage 4 bud 3 days after treatment. Arrows indicate the abscission zone (AZ) separation layer, which is clearly formed in B and C.

Treating the abscission zones directly with ethephon allowed a better interpretation of the effect of ethylene on the calyx and peduncle abscission zones. The ethephon application to the calyx abscission zone caused it to develop the same symptoms of discoloration and swelling one day following the treatment previously noted with ethylene exposure. After the ethephon application to the calyx abscission zone on Stage

2 buds, the separation layer in the peduncle abscission zone was clearly formed by the fourth day (Fig. 5-11). The actual separation took place after 6 days which was 2 days later than observed when buds were exposed to ethylene in Exp. 3-1. The slower response in abscission zone development may be due to the application method of ethylene.

When only the peduncle abscission zone was treated with ethephon, the separation layer did not form (Fig. 5-12) and the bud continued to develop normally through flower opening and senescence. The peduncle abscission zone did not appear to be affected by ethephon treatment; thus, suggesting that the peduncle abscission zone is not mediated by ethylene.

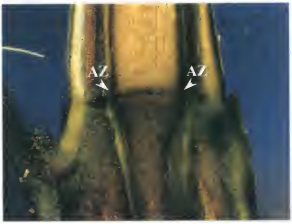

Figure 5-11: Longitudinal section of the peduncle abscission zone 4 days after ethephon treatment to the calyx abscission zone on a Stage 2 bud. Arrows indicate the abscission zone (AZ) separation layer

In this investigation, it was noted that following senescence the calyx abscised at the calyx abscission zone and 4 to 5 days later the remaining pedicel abscised at the peduncle abscission zone. Results obtained from these 3 experiments show that the calyx abscission zone of *H. rosa-sinensis* is preformed early in bud development, but does not become functional until induced by senescing of the petals or ethylene exposure.

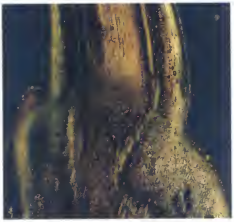

Figure 5-12: Longitudinal section of the peduncle abscission zone 4 days after ethephon treatment to only the peduncle abscission zone of a Stage 2 bud. A separation layer is not visible.

Whereas, the peduncle abscission zone is not preformed and forms only after the calyx abscission zone is activated or removed. Results also clearly showed that the separation of the peduncle abscission zone was not mediated by ethylene and the only time it was activated was when the calyx abscission zone has become functional or separated.

CHAPTER 6
CONCLUSIONS

Shipping potted Hibiscus is a commercial problem due to abscission of flowers and buds, which reduces the quality of the plants. The objective of this work was to document bud abscission in *H. rosa-sinensis* during shipping by defining the temperature and durations necessary to cause the effect. Also the objectives were to determine the effect and the role of ethylene in bud abscission and to understand the anatomical mechanism of the calyx and peduncle abscission zone during abscission.

Plants shipped at high temperature (25°C) and for longer durations (6 days) abscised all their Stage 1 and 2 buds compared to plants shipped at lower temperatures (13 and 18°C) and shorter duration (2 days). Results confirmed the findings of others that *H. rosa-sinensis* is very sensitive to exogenous ethylene and that ethylene accelerated bud abscission. Stage 1 and 2 flower buds were affected more by low ethylene concentrations (0.2-0.3 $\mu L.l^{-1}$) as they prematurely dropped, while the remaining buds developed through flowering and senescing. In contrast, at a higher concentration (1 $\mu L.l^{-1}$) the larger buds drop first followed by the smaller buds.

The use of ethylene blockers, silver thiosulphate (STS), aminoethoxyvinyl glycine (AVG) and 1-methylcyclopropene (1-MCP) showed that bud abscission during shipping in *H. rosa-sinensis* is mediated by ethylene. AVG delayed the onset of bud drop during shipping, as the number of buds retained (or reaching flowering) was greater on plants treated with AVG. At higher temperature (25°C) and for 6 days, AVG reduced Stage 1 and 2 bud drop. AVG completely prevented abscission when plants were shipped at lower temperature (20°C). Silver thiosulphate prevented bud abscission when the plants were shipped at lower temperatures. During shipping the number of buds abscised from plants pretreated with 1- MCP was similar to control plants. 1-MCP was also tested over time when plants were pretreated with 1-MCP then exposed to ethylene. Results showed that 1-MCP completely prevented ethylene induced bud abscission when exposure to ethylene immediately followed the 1-MCP treatment. However, flower bud abscission occurred when ethylene exposure was 2 days following the 1-MCP treatment. These results led to the conclusion that new receptor sites are being formed while the plants are in shipping and these sites are not protected against ethylene produced during the stress of shipping.

To address changes on abscission zones, the final study looked at longitudinal sections of calyx and peduncle abscission zones. Sectioning of the calyx abscission zone was done for all bud stages on greenhouse grown plants. Whereas, sectioning of the peduncle abscission zone followed ethylene treatment and the removal of the bud above or below the calyx abscission zone. Observations showed that the calyx abscission zone was preformed (i.e. appeared as a morphological feature at an early developmental stage) and cell division continued up to the time of abscission. The calyx abscission zone was induced by ethylene, which caused premature formation of the separation layer in the abscission zone. On the other hand, the peduncle abscission zone formed only when abscission was induced in the calyx abscission zone. During normal development, the flower bud senesces and after a few days the calyx drops at the calyx abscission zone leaving the peduncle intact for 4 or 5 days before it too drops. When the bud was removed below the calyx abscission zone the remaining portion of the peduncle dropped

4 days later. The peduncle abscission zone appears to be induced only after the calyx abscission zone has functioned and abscised (following senescence of the petals) or when removed. The peduncle abscission zone was not induced by ethylene.

The role of ethylene in bud abscission of *H. rosa-sinensis* during shipping was established in this study, in agreement with previous studies (Høyer, 1986; Thaxton et al. 1988); however, differences in developmental bud stage abscising was found. Neither Høyer (1986) nor Thaxton et al. (1988) specified the pattern of bud drop during shipping or when exposed to ethylene. The use of ethylene blockers may reduce the damage caused by shipping, but only when shipping is at lower temperatures. Further study needs to be done on 1-MCP and temperature to determine if at lower temperature 1-MCP is capable of blocking ethylene and reducing shipping induced abscission. Even though AVG was reported to inhibit or reduce bud loss during shipping, commercially it has not received much attention since AVG cannot block exogenous ethylene. Although during shipping most of the ethylene comes from the plants, exogenous ethylene from smoke and fumes may damage the plants

Studying the abscission zone anatomically allowed us to have a better understanding of the process of abscission in *H. rosa-sinensis*. With recent technologies, such as gene transformation, a possibility may arise to block the function of the calyx abscission zone, which in turn will stop the peduncle abscission zone from abscising. At present several approaches could be taken to reduce shipping damage such as the use of ethylene blockers (STS or AVG) and regulating the shipping temperature, especially when shipping for longer durations.

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BIOGRAPHICAL SKETCH

Fatma A. Al Saqri was born in Izz, Sultanate of Oman. She completed her high school education at Sultan's School, Muscat, in June 1986 and entered Sultan Qaboos University for her bachelor's degree. In June 1990, Fatma received her Bachelor of Science in plant science and started working at the Diwan of Royal Court, Department of Agriculture and Veterinary Services, in September of that year. She was appointed to the Royal Nursery where she gained experience in the propagating and growing of plants, as well as managing greenhouses.

In August 1993 she was given a scholarship and entered Nottingham University, UK where she completed her Master of Philosophy in horticulture. Upon competition of her master in December of 1995, she returned to Oman and returned to work as the head of the Royal Nursery where she took on the responsibility of planning and managing the operations of the entire nursery. In August 1998, she was granted another scholarship to pursue her doctoral degree in horticulture at the University of Florida. In summer of 2000 Fatma joined the Environmental Horticulture Department to investigate the causes of floral abscission during shipping of *Hibiscus rosa-sinensis*, an important and popular flowering potted plant. She will be returning to Oman to assume new duties at the Diwan of Royal Court following the successful completion of her doctoral degree.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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